# NATURE

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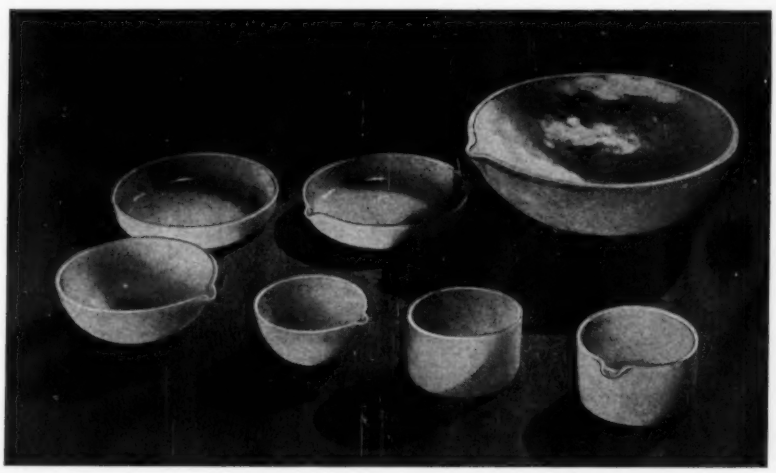

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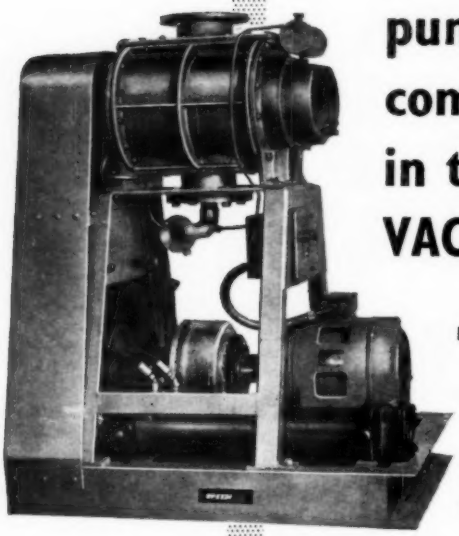

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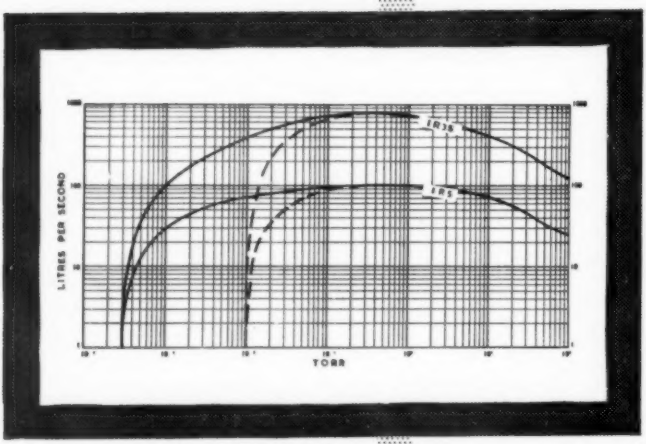


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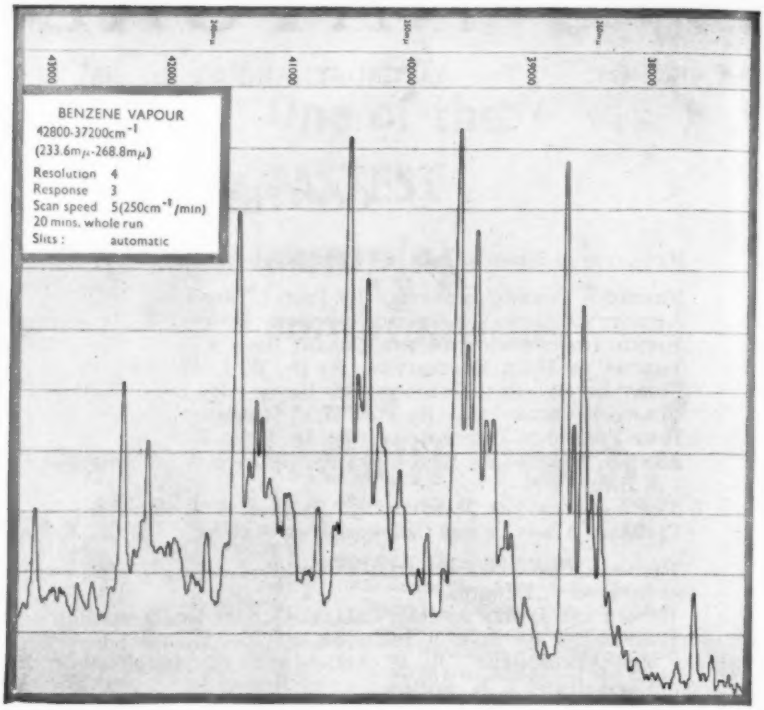

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# MANAGERIAL RESOURCES : AN INTERNATIONAL SURVEY

SOME reasons for Britain's continuing difficulties with her balance-of-payment problems have been revealed in three recently published works. An American book presents an international picture of management in a number of industrialized countries[1]. A British book deals particularly with the management scene in Britain[2]. The third is a comparative study by an American don who has looked critically at American and Russian industry and, after a year as a Fulbright Scholar in Britain, has produced a book which points to the inescapable conclusion that British industry will remain hamstrung until there is a marked improvement all round in the quality of her executives.

The seesaw of import–export figures with every other year producing too little to cover long-term overseas investments has been with us since the War. Exhortation for more productivity to give us more exports has led up to the fourth balance-of-payments crisis during the past decade and has once more caused the Chancellor of the Exchequer to apply the brakes to industrial investment. The overall result has been a slow rise in British productivity which has been insufficient to pay for our imports, the improved standard of living which should accompany productivity, and to leave enough for essential overseas investment. The tragedy for Britain is that other European countries have been able to steer clear of balance-of-payment difficulties. An authority like Colin Clark has confirmed that increases in productivity of most industrialized countries have exceeded those of the United Kingdom[4]. The rise over the past year has still not been enough to prevent the Chancellor calling a halt to expansion.

Many of our difficulties lie, of course, within the political, economic and social fields. But from Harbison and Myers's analysis of management in the industrial world, a considerable part of our trouble is bound up with the quality and distribution of men occupying top and middle management positions in our industrial organizations.

Harbison and Myers first define the bases on which their comparisons of management are made, and then, with expert help from various countries, go on to examine the nature of management as an economic resource, as a system of authority and as a class, as well as the development of managerial resources in different industrialized countries.

Compared with countries like India, Egypt, Chile and Israel, which are less developed industrially, Britain, in common with other advanced industrial nations like Japan, Germany, Sweden, the U.S.S.R. and the United States, possesses reserves of actual and potential management talent which helps them to maintain their industrial supremacy. That this position is not likely to go without challenge is shown by Egypt, where institutions for the generation of high-talent man-power are quite advanced. In contrast to France, Italy and many of the newly developing countries, Egypt's industrial progress is not retarded by a conservative patrimonial *élite* and its managers . . . "are receptive to new ideas". Despite the warnings and forebodings of British politicians, for example, in 1958 the all-Egyptian management of the Suez Canal Authority was handling 25 per cent more ship traffic than "its predecessor foreign management handled before 1956. At the same time, the total number of white-collar, technical, professional and managerial positions has been reduced by 23 per cent". In Israel, a vigorous, young and aggressive managerial class has sprung up with growing social prestige.

For Britain, management comparisons with other advanced industrial countries are more revealing. In Japan, concentration of industrial power in the hands of the *zaibatsu* has led to the remarkable development of human resources in general and high-talent man-power in particular. The vast industrial empires of the *zaibatsu*, motivated by a nationalistic patriotism, have been successful in building managements which are imbued with the spirit of hard work, perseverance and unfaltering loyalty both to the organization and the dynasty which controls it. In the larger firms there is an almost feverish interest in new techniques, and to-day Japan is probably one of the few countries which could easily export high-level man-power resources without straining its economy; entry into the ranks of professional management is based primarily upon formal education.

The West German achievement is well known; there is no adequate parallel to the transformation of West Germany since the War. Between 1950 and 1957 the gross national product was more than doubled; the British increased slightly.

Like his counterparts in the United States, the U.S.S.R. and Japan, on the solid basis of prestige, power and wealth, the German manager vies for prominence among the members of other high-status groups. A significant difference between the German system and the British is that, in Germany, the top-level man, the *Unternehmer*, has real authority and direct interest in the business he controls, and is so free from day-to-day executive activities that he is able to forecast, plan and direct the policy of the company. Few boards of directors in Britain are able to function in this way, although our system of having a good sprinkling of executive directors has much to commend it.

The great difference between German, Russian and American management and that of the United Kingdom lies in the educational background of the men and women who become managers. In 1955, for example, at least 31 per cent of German top management had university degrees; the largest

single group consisted of graduates from the *Technische Hochschulen*. The position is much the same in the United States and the U.S.S.R. In the U.S.S.R. the change has been from a peasant, agrarian economy to a high level of industrial achievement since the introduction of the first five-year plan three decades ago. This has not entirely been due to the extreme use of totalitarian methods, concentration on heavy industry and the sacrifice of short-run consumption to facilitate the investment needed for long-term development. The main emphasis in the Soviet Union during the period has been on the development of well-qualified management[5]; the economic results are well known. If nothing else, the Soviet experience provides strong support for the belief that industrialization requires that access to important managerial positions be based on objective standards of competence rather than 'connexions'. Even in their doldrum days of the '30's, the Soviet leaders showed their long-sightedness in insisting that the British, German and American 'foreigners' should be used for training their own men for the future rather than producing results at the time.

The American position needs little underlining; the proportion of personnel of high talent among American management is the highest in the world. In contrast to much of British industry, competence and performance on the job have become the tests by which men are judged, although these reflect not only innate capacities but also prior education; between 50 and 60 per cent of executives have university degrees; and, because of the status which management enjoys in social and economic life, the recruitment of talented young men destined for managerial positions is easier than in most countries. A major difference between American and most other industrialized countries is in the emphasis she has given to the teaching of management subjects in her business-administration schools. Formerly, these were mainly concerned with provision for undergraduates. Since the Second World War, fifty colleges and universities have developed full-time postgraduate courses for industrial executives. Although some of the work done in these postgraduate schools might be rated as shoddy, Prof. Norman Hunt, of the University of Edinburgh, has written that "work of the highest quality both in research and teaching is being undertaken: much of it would stand up to the scrutiny of the most critical of us". The fine work being done in business-training schools is complemented by executive development programmes inside industrial organizations themselves. There is little doubt that American managers are, at present, the best trained in the world. Has this some connexion with productivity?

The British position has been admirably described by McGivering, Matthews and Scott[2], and, although their conclusions must be treated with some reserve, the impression they create does not lead to the belief that Britain can hope, in the near future, to match the examples of Japan, West Germany, the U.S.S.R. and the United States in their drives for productivity.

There are a number of reasons for British weaknesses and difficulties. It is now well established, for example, that the higher the degree of mechanization and the more sophisticated the environment in an industrial organization, the higher will be the ratio of managers required. This is necessarily accompanied by increasing specialization without automatically increasing the overall size of the organization; as Joan Woodward has indicated, "*specialization* technology rather than size *per se* is most important in promoting specialization within management". Because they find it difficult to see the place of the specialist and because they fear his challenge in the struggle for promotion, many senior executives have done their utmost to resist specialization albeit to the detriment of their companies.

Other factors tend to maintain centralized authority and control. "Firms which are owned by families, or in which the major share-holding interest is held by some other single group, are notoriously loth to expand", if this would lead to any loss of control or delegation of authority. Delegation, too, with all its advantages, can be achieved with greater confidence when adequate measures of the delegatee's performance exists. "British management . . . is backward in the employment of techniques of work measurement, cost accountancy and budgetary control." There is also a deep-rooted belief among many senior executives that the higher management skills and especially those which go with 'leadership' are qualities which they possess but are denied to their subordinates; they are believed to accompany a public school education.

There are also factors to indicate that British industry continues to suffer because of social distinctions. Evidence is accumulating that "the rank order of advantageous categories . . . shows the superiority of an Arts degree (Oxon. or Cantab.) or major public school education over an Arts degree (Redbrick) or lesser public school or grammar school attendance". One investigation has shown that there is a direct correlation between social origin and salary; consciousness of social handicaps appear to be more prevalent in family firms and in firms recruiting largely from universities.

A major weakness is in the attitude of many managers towards productivity itself. In Great Britain productivity has frequently been interpreted as low-cost production to yield high returns on a steady market. American companies with manufacturing units in Europe have been quick to exploit their national habit of driving existing plants to achieve greater output without sacrificing quality or increasing costs; Britain has been slow to copy them.

Unfortunately, too, and this may be bound up with the social class background of many British senior executives, the trade unions are still regarded as enemies who must always be fought; the trade unions have responded to the attack, and the joint enterprise of American management and labour for

increased productivity with sharing of rewards has not been repeated to the same extent in Britain.

How, then, can Britain hope to achieve a standard of productivity which will enable her to sell exports increasingly in direct competition with other advanced industrialized nations? The quality of management must be raised by increasing the standard of the recruits. Graduates must be used, and more scientists and technologists must command influential positions in the board room. Standards must be set against which performance can be judged. Men must be selected for their competence and not for their social background; there is little doubt that the talents of Great Britain are not being fully used, and perpetuation of the existing class system could lead to national disaster. Training schemes in firms must be developed to produce executives capable of making decisions as well as technicians and craftsmen to support them. Active trade unions should be encouraged to help in the drive for productivity. Above all, the supply of scientists and technologists must be stepped up; a heavy burden rests upon the Ministry of Education and local authorities.

All this will be vitiated, however, unless public opinion outside and inside industry drops its complacency and builds up a climate of opinion in which young men of talent can be given opportunities for making an attempt to raise productivity to standards not yet contemplated. Until this occurs, we can expect to have balance-of-payment difficulties with continuing regularity.

[1] Management in the Industrial World: an International Analysis by Frederick Harbison and Charles S. Myers (McGraw-Hill, London, 1959).

[2] Management in Britain: a General Characterization, by I. McGivering, D. G. J. Matthews and W. H. Scott (Liverpool University Press, 1960).

[3] The Red Executive, by David Granick (Macmillan and Co., Ltd., London, 1960).

[4] The Conditions of Economic Progress, by Colin Clark (Macmillan and Co., Ltd., London, 1957).

[5] Engineering Education in Russia, by S. P. Timoshenko (McGraw-Hill, London, 1959).

[6] Management and Technology (H.M.S.O., 1958).

## NEWTON'S CORRESPONDENCE

### The Correspondence of Isaac Newton

Edited by Prof. H. W. Turnbull. Vol. 1: 1661–1675. Pp. xxxvii+468+7 plates. (Cambridge: At the University Press, 1959. Published for the Royal Society.) 147*s*. net.

THE publication of the first volume of a critical edition of Newton's correspondence is an important event in the domain of history of science. Few periods in the development of human culture offer a more instructive and fascinating field of study than that which saw the birth of modern science. The basic data for such a study, in the form of letters and manuscripts as well as printed books and papers, exist in great abundance, and allow one—especially the letters, with their more personal and intimate communications—to form a vivid picture of the pioneers' adventurous exploration of Nature and of their struggle for the vindication of rational thinking and the establishment of proper methods of scientific analysis. Much of this material has long been available to scholars, above all in the monumental editions of the works and correspondence of Galilei, Descartes and Huygens. In this connexion, the insufficiency of the existing edition of Leibniz's scientific papers, but still more the lack of any coherent publication of Newton's works, has always been felt as a most serious inconvenience, which is still to this day hampering a really exhaustive investigation of some of the most critical phases in this momentous development.

On the Continent, the task of editing the works of great men is regarded as an undertaking of national concern, deserving the support of the Government as well as that of private bodies and institutions. The traditional aloofness of the British Government with respect to cultural matters has had no small part in the long neglect suffered by the Newton papers; but, without unkindness to a venerable institution, it must be said that the Royal Society was also very late in awakening to its responsibility. Prof. E. N. da C. Andrade's introduction to the present volume, in reciting the story of the Society's handling of the matter over many years, has a sad tale to tell of procrastination and inefficiency. Even now, when a most auspicious start has at last been made, our satisfaction is tempered by the disappointing confession that the Society does not feel able to undertake more than the publication of the correspondence; for a critical edition of Newton's great books and of his numerous and still incompletely surveyed manuscript notes and papers, scholars with the necessary leisure and competence, according to Prof. Andrade, are not to be found. In making this excuse he seems to lose sight of the vigorous growth of the modern school of scientific historians; anyhow, it is happily belied by the fact that at this moment, in the United States, two distinguished historians are actually preparing a critical edition of the "Principia", while the study of Newton's unpublished papers is also being actively pursued.

The time is not for incrimination, however, when we are presented with such a splendid achievement as this publication of Newton's correspondence; if the further volumes keep the same high standard, a monument of scholarship rivalling those recalled above will have been raised to Newton's memory. In fact, we are given more than just the letters: partly to atone for the renunciation to edit all the manuscripts, a selection of the latter, namely, those more closely connected with the correspondence, is also included. All texts are accompanied, in conformity with the exacting requirements of modern historical criticism, by the relevant information about their nature and provenance, and by abundant notes elucidating their contents. As regards the latter, some minor blemishes are inescapable—I hope to publish elsewhere a list of the corrections and additions which I noticed during the perusal of the volume—but on the whole the notes are excellent, especially on matters of mathematics and optics. The only serious shortcoming is the lack of accurate bibliographical descriptions of the books quoted (for example, in the form adopted in the Huygens edition); this desideratum could perhaps still be fulfilled in the future volumes. For the accuracy of the text, we must of course trust the editors, and we have certainly no reason not to do so: on internal evidence I have only detected two trifling textual errors (in French texts). The historians of science are indeed deeply indebted to Prof. H. W. Turnbull and his collaborators, the late Mr. W. H. Robinson and Dr. J. F.

Scott, for accomplishing their important task in such a painstaking and skilful fashion.

If we now turn to the contents of this first volume, which covers Newton's youth and his first creative period, during which most of the optical work was done and the new ideas about mathematical analysis and the theory of gravitation took shape, I cannot say that it discloses any striking novelty. Most of the letters and documents had already been published, and the fuller view we now gain by their more complete presentation can only increase our respect for the judgment of the Victorian scholars, Edleston, Rigaud, Brewster, who made the previous selections; it is comforting to realize, in particular, what careful and sagacious use good old Brewster, with all his oddities, could make of his sources. Nevertheless, renewed studies of these sources in their authentic and complete form cannot fail to reveal interesting new details, enlivening the picture of the times and deepening our understanding of the processes of discovery. Especially valuable in the latter respect is the publication of numerous documents illustrating the little-known relations between Newton and Gregory; it is the editor's great merit to have directed attention to them.

Reading such correspondence, in the chronological order in which it is presented, is like listening to a symphony the various themes of which successively appear and interlace, evoking the hero's growth and deeds against the background of his time and environment. In this respect, the comparison of the Huygens and Newton papers during the same span of their lives (three volumes to one!) illustrates in a most telling fashion the difference between the social classes to which they belonged and which supported and influenced their work. Of Newton's rustic youth scarcely any record remains, whereas there is a wealth of documents picturing all the stages of the opulent Huygens children's refined education and their development into sophisticated and slightly snobbish gentlemen. As soon as Huygens's precocious genius shot its first buds, he was admitted with demonstrative honour to the choicest circles of virtuosi; the poor Cambridge scholar had to be content with the less-glamorous, but perhaps more substantial esteem of plain people like himself, engrossed in the business of trade and administration, but compensating the lack of elegance in their intellectual demeanour by seriousness of purpose and a keen sense of reality.

It is highly instructive to watch how John Collins puts young Newton's mathematical skill to the test by proposing to him problems of annuities and related algebraic questions: of such obscure birth is modern analysis! The next theme, recurring through a great part of the volume, is the construction of a reflecting telescope; it is in this work as well as in their mathematical research that Newton and Gregory followed parallel ways, of which they became acquainted through their common correspondent, the always friendly, sometimes officious Collins.

Then comes the dominant feature of the period spanned by the volume under review, the great work on the theory of colours and the nature of light, which is here very fully documented. We may follow in minute detail the famous controversy to which Newton's investigations gave rise, so startling did his new method of reasoning by induction from experiment appear even to men like Huygens and Hooke. However, a careful reading of Hooke's objections, now for the first time available *in extenso* (I allude to the important new document No. 71), reveals that the latter had spotted a real weakness in Newton's argument, namely, his too rigid assertion of the permanent character of the homogeneous coloured rays. In this light, Newton's famous conciliatory letter to Hooke (No. 154), which has hitherto been presented as an example of generosity, cannot fail to strike one, on the contrary, as unpleasantly condescending and arrogant. Without denying Oldenburg's part in embittering the quarrel, one feels that Newton's own obstinacy and unwillingness or incapacity to understand Hooke's point are amply sufficient to account for it.

It is well known that the conception of universal gravitation goes back to the same period of Newton's life, when, as he will later declare, "I was in the prime of my age for invention". Yet, from this time we have only one document (No. 117) of extreme interest, recently brought to light by Dr. A. R. Hall: it is the first appearance of the mightiest theme in the Newtonian symphony, which will unfold itself throughout the further, eagerly awaited instalments of this magnificent publication. L. Rosenfeld

## ASTROPHYSICS OF NON-STATIC SYSTEMS

### Moving Envelopes of Stars

By Prof. V. V. Sobolev. Translated by Sergei Gaposchkin. Pp. xiv+106. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1960.) 38s. net.

THE astrophysicist trying to infer the development of astronomical systems from the available observations is generally like someone trying to imagine the story of a film from a few 'stills' shown outside a cinema. Most astrophysical theory is concerned with such an exercise. However, the astrophysicist is occasionally afforded the advantage of a 'trailer', when he actually sees some change occurring in the heavens as in a nova eruption or in cases of matter obviously streaming away from stars. But a trailer is usually concerned with the more colourful episodes in a story, and a special skill is needed for correctly imagining the whole film from it. The present book is concerned with the analogous exercise in astrophysical theory.

The contents of the book, however, are easy neither to describe nor to assess. This is mainly because, as Prof. Sobolev says, he leaves the general account of the subject to be found in text-books and here presents only his own results, seemingly in much the form in which they were originally published in Russian journals. At any rate, he states the purpose very clearly as "to give a theory of radiative equilibrium of a moving medium and to apply it to the moving envelopes of stars. Because of the breadth of the problem, only the basis of the theory and its most immediate applications are given here".

The account is concise and free from unnecessary complications. In fact, it is so concise that only someone currently working in the field is likely to be able to appreciate its full value. But even someone such as myself whose ventures into the field were some time ago, can realize that Prof. Sobolev has made important advances in the theory and its applications, and that Dr. Gaposchkin has done good service by making them better known by translating them into English. W. H. McCrea

# SOUND TRANSMISSION IN THE OCEAN

## Introduction to the Theory of Sound Transmission with Application to the Ocean

By C. B. Officer. (McGraw-Hill Series in the Geological Sciences.) Pp. viii+284. (London: McGraw-Hill Publishing Company, Ltd., 1958.) 77*s.* 6*d.*

THE author of this volume is associate professor in the Department of Geology, The Rice Institute, and associate in geophysics, Woods Hole Oceanographic Institution. These facts are mentioned to indicate that the book is of interest not only to physicists who are concerned with sound propagation in the sea, but also to geologists and geophysicists who are concerned with the wider aspects of the subject.

The propagation of sound in the ocean, in shallow seas, and in the Earth beneath the sea has important implications which concern a wide field of science, namely, physics, geophysics and oceanography.

In the preface the author directs attention to the fact that the preparation of the book has been sponsored ("encouraged and supported") by the Office of Naval Research and the U.S. Bureau of Ships, thus indicating its importance to those concerned with the applied aspects of a study of sound propagation in the ocean.

Reference is also made in the preface to many well-known American workers in this field, for example, J. B. Hersey, M. Ewing, J. L. Worzel and others.

The subject-matter of the book is divided into six chapters. The first of these deals with general wave theory for an elastic medium, including the cases of waves of finite amplitude and shock fronts. The second chapter is concerned with the 'ray' and 'normal-mode' theories of sound propagation, with refraction of a dispersive wave-train, and with prescribed boundary conditions. In Chapter 3, these considerations are applied to the case of transmission in shallow water where interference due to multiple surface and bottom images has to be taken into account. The fourth chapter deals with the case of deep oceanic water, where the bottom is not involved. In this case refraction due to temperature and pressure effects on sound-velocity is considered. Chapter 5 is concerned with 'reflectivity' of boundary layers, reflexion from three layers, and the interpretation of echo-sounding records and of geophysical reflexion and refraction profiles. The concluding chapter, "Attenuation", covers absorption in a viscous fluid, scattering and diffraction, and reception by a linear array.

Chapters 3 and 4, which deal respectively with the transmission in shallow water (inshore seas) and in deep water (the oceans), show the difficulties involved in attempting to arrive at a reliable theoretical solution of wave propagation in the sea. Consideration must ultimately be given to many factors, for example, the effects of depth and configuration of the sea-bed, the physical (acoustic) properties of the sea-bed material, the sound-velocity structure of the water (temperature- and pressure-gradients as they influence velocity of propagation), the shape of the sea-surface (waves and swell), the presence of sound-scatterers in the water, and so on. As the author very truly states, "A complete formulation with all possible variations of the above environmental conditions would be very difficult to obtain." The theoretical complications in shallow water transmission due to multiple reflexions between sea-surface and sea-bed are dealt with on both 'ray' and 'mode' theories, but there is clearly much more to be done to predict the sound-distribution in a shallow sea even under the most 'ideal' theoretical conditions of isothermal water and a flat bottom parallel to a flat sea surface.

The problem is a difficult one, and Prof. Officer has made a praiseworthy effort in presenting the theoretical aspect in a stimulating and readable fashion. As an introduction to the subject the book will serve a very useful purpose in arousing interest in physicists, geophysicists and oceanographers alike.

The book is well written, illustrated and produced and can be recommended to all those interested in this difficult but fascinating study of sound transmission in the sea.

A. B. WOOD

# THEORY OF HIGH EXPLOSIVES

## The Science of High Explosives

By Prof. Melvin A. Cook. (American Chemical Society Monograph Series, No. 139.) Pp. xv+440. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1958.) 180*s.* net.

THIS book is a valuable addition to the rather sparse literature on the subject of explosives. Its scope is wide; but, as the title suggests, it is concerned more with the basic physical and chemical principles underlying the behaviour of explosives than with practical applications. Nevertheless, there are parts that will be of general interest, in particular the chapters describing the modern explosives industry and the explosion properties and blasting action of high explosives.

Perhaps the heart of the book is formed by those chapters dealing with the mechanism of initiation and propagation of detonation and the accompanying phenomena. Particular attention is paid to the nature of the reaction zone in detonation waves, and the author considers that the classical treatments of Zeldovich, von Neumann, Doering, and others should be modified to include the notion of heat conduction. In addition to claiming that this modification gives a better explanation of the observed distribution of parameters in the reaction zone (for example, the evidence for the existence of a pressure 'spike' predicted by the original theories is inconclusive), he says that it explains what happens to the critical enthalpy that is necessary to initiate detonation. He considers that this enthalpy is contained within the reaction-zone and propagated as a heat pulse. The author claims to have obtained photographic evidence of a heat pulse during the growth to detonation of an initially non-reactive shock-wave in the liquid explosive nitromethane, but unfortunately the features in the original colour photographs to which he ascribes considerable significance do not show up well in the black-and-white reproductions. Also discussed in some detail are the respective theories of non-ideal detonation due to Jones and to Eyring and others. An alternative theory, called the geometrical model, is put forward which asserts that not only must lateral losses, which affect only the equations of continuity, be taken into account but also that only part of the reaction zone lies between the detonation front and the Chapman–Jouguet plane, so that not all the chemical energy released is available for support-

ing the detonation wave. As mentioned in the book, this theory is essentially the same as that proposed by different authors as long ago as 1928 but not then generally published.

There are useful chapters on the instrumentation used in modern explosives research and on the effects of air and ground blast waves, and considerable space is given to shaped charges and to the behaviour of solids under explosive attack. Perhaps the most disappointing parts of the book are those dealing with the blast waves produced in gases by detonating explosives; no mention is made of the modern approach based on the methods of fluid mechanics and the descriptions are derived from somewhat questionable qualitative assumptions.

The book is not easy to read; in the attempt to be comprehensive there is a tendency in places to deal too briefly with difficult physical concepts and this fault is aggravated by the widespread use of symbols as a shorthand method of writing. Even though the symbols are clearly defined in an appendix it is irritating to have to be continually referring to it. However, it may be said that this is a reference volume for the research worker rather than a textbook, and as such its wealth of detailed information and exhaustive lists of references make its appearance welcome.

W. L. Murray

## STORY OF CHEMICAL INDUSTRY IN BASLE

### The Story of Chemical Industry in Basle

Pp. 234. (Published by CIBA Limited on the occasion of its 75th Anniversary.) (Basle: CIBA Limited, 1959.)

THE title of this work is far too modest. In 233 beautifully printed and illustrated pages the history of chemistry is set out in such a way as to give Basle its rightful place in the fields of drugs, plastics, textiles and dyeing. Although Ciba Ltd. are the publishers, full credit has been given to the firms of Sandoz, Geigy, and Hoffmann La Roche, as well as to other firms and individual scientists throughout the world, for their contributions to science and technology.

The first part describes the founding of the first Universities in Paris (by Bartholomew the Englishman in 1231), Oxford, Salerno and Montpellier. The influence of the Guilds is noted as is also the contribution to the Renaissance made by the monasteries. In the fourteenth century the Dominican monastery in Basle possessed works by Avicenna, commentaries on Aristotle, Latin translations of the works of Galen and the medical works of Albert the Great (usually referred to in English books as Albertus Magnus). In 1460 the University of Basle (modelled on those of Paris and Bologna) was founded and eight years later the printing of books commenced in Basle.

The University of Basle received a refreshing blast of fresh air in 1526 when Paracelsus occupied the chair of medicine for a few months. As every student of chemistry knows this rebel publicly burnt the works of Galen and Avicenna and scoffed at the alchemists, stating that medicine, not gold, was the true aim of alchemy.

The story continues to the eighteenth century, and the age of enlightenment. Voltaire, Diderot, Nicholas Leblanc, Edmund Cartwright, James Watt, Adam Smith, Montesquieu and Rousseau are just a few of the famous in all branches of philosophy who appear in the pages of this remarkable book. Next we read how refugees from the Reformation and the counter-Reformation transplanted luxury industries to Basle.

The second part of the book ("The Development of the Chemical Industry in Basle") commences by showing that chemical industry grew from the co-operation between the trade in crude drugs and colouring matters, the apothecaries' shop, and the gas works. In a short history of the ancient drug trade drugs which are also colouring matters (for example, saffron, buckthorn), are mentioned, as well as many others, some of which are illustrated in colour. Surprise is expressed that until the nineteenth century the whole drug was considered essential and no one thought of searching for pure 'active' principles. Extraction of 'principles' soon became commercially popular and in 1857 J. R. Geigy and U. Heusler built a plant in Basle to extract dye-woods. The silk ribbon industry in Basle naturally attracted dyers to the neighbourhood. In 1859, three years after Perkin's discovery of mauveine, Geigy switched his attention to the manufacture of synthetic dyes, starting with the preparation of fuchsine. Also in 1859, Clavel commenced the manufacture of fuchsine in Basle and his firm eventually became Ciba (a name formed from the initial letters of the Society for Chemical Industry in Basle).

The factors leading to the rapid growth in chemical industry in Basle are described. These are briefly (1) the ease with which coal-tar could be obtained from England, France and Germany, (2) the advantage of being able to dispose of waste products in the Rhine, (3) plenty of capital from the older industries, (4) the local market for dyes provided by the silk ribbon industry, (5) the socio-political freedom arising from the unification of the Swiss cantons into a federal State, and (6) the rapid rise in the population (and hence the labour supply) of Basle.

Dyestuffs are next dealt with in some detail, and I have space here only to mention such names as Liebig, Perkin, Hoffmann, Verquin, Graebe and Liebermann, Caro, Peter Griess, Martius, Walter, Baeyer, Sand-Meyer and Heumann.

The history of the scientific pharmaceutical industry commences in this book with the founding of the first institute for experimental pharmacology by Buckheim in Estonia in 1849. Then follows the bacteriological and antiseptic discoveries of Semmelweis, Pasteur, Lemaire, Lister and, last but not least, Socin in Basle, who in 1871 was using phenol for wounds. Then on to Robert Koch's *in vitro* cultivation of bacteria and the work of Ehrlich on the application of dyes and arsenicals to medicine. Among the newer bactericides we read how Prontosil (Domagk 1935) led to 'Cibazol' (Ciba—1938, better known to us as Sulphathiazole). In 1950 the La Roche group produced Isoniazide (for tuberculosis) and Ciba in 1958 introduced 'Ciba 1906' for leprosy.

Turning to drugs derived from natural precursors we are told the story of Nupercaine (Ciba 1929), Dolantin (1940) and Cliradon (Ciba 1949) and of Butazolidine (Geigy). (The latter derived from quinine in the mistaken belief that antipyrine is a quinoline derivative.)

Similar treatment is given for very many other organic medicaments: Ergotamine (Sandoz 1918), Coramine (Hartmann of Ciba, 1918), Priscol and Privine (Ciba 1939), and the isolation of Reserpine in 1952 (Ciba). Vitamin C was first synthesized by Reichstein in 1933 and within two years La Roche

were manufacturing it. In the 'thirties Ciba helped to isolate progesterone and later worked with Reichstein on cortisone.

Part 2 finishes with a historical account of plastics, from ancient ceramic arts via gun-cotton (Schoenbein of the University of Basle in 1845), celluloid, the Chardonnet denitrification process, to the important modern epoxy resins of to-day such as Ciba's 'Araldite'.

The plain grey board covers of this book give no hint of the wealth of material within. Beautifully printed on high-quality paper with hundreds of illustrations, dozens of which are in beautiful colours, it stands in a class of its own.

I have noticed only one error of fact (p. 123). The dibromoanthraquinone which Graebe and Liebermann hydrolysed to alizarin was, in fact, the 2 : 3 dibromo compound and not the 1 : 2 as described, although this piece of 'research luck' was not discovered until several years after the event. A few expressions strike the English reader as unusual, for example, "in six years the price of quinine *dove* from 1·3 to 2·5 florins per oz.", "diazo dyestuffs" (we say 'azo dyes'), and the use of the word 'somnifacients' for what we (with perhaps less Latin and more Greek) call 'hypnotics'.

This work reflects great credit on Drs. Huber and Menzi, who wrote the text, on Drs. Wilhelm and Kappeli, who had the idea of thus celebrating Ciba's seventy-fifth anniversary, on the photographers and blockmakers, and on all those concerned with the book. All who are able to obtain a copy are indeed to be envied.

L. K. Sharp

## QUANTUM CHEMISTRY

### Quantum Chemistry

Methods and Applications. By R. Daudel, R. Lefebvre and C. Moser. Pp. xiii+572. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1959.) 14.50 dollars.

IT is an interesting exercise to speculate on the probable contents of a new book on quantum chemistry. One can be fairly sure that there will be a discussion of the particle in the box, the hydrogen atom, the hydrogen molecule-ion, the hydrogen molecule itself and possibly methane, ethylene and benzene. But for the serious student of the subject it is the rest of the contents which will excite his main interest. The books entitled "Quantum Chemistry" which are already on the market differ very greatly in what their authors have seen fit to add to this rather pedestrian list of topics. The book by Eyring, Walter and Kimball makes much use of the theory of groups in the interpretation of spectra and is one of the best elementary sources for information about electromagnetic phenomena; on the other hand, in that book there is almost no discussion of the diverse and sophisticated facts of chemical reactivity. The book by Pitzer covers widely the basic principles of chemical physics, and lays considerable stress on statistical mechanics and molecular dynamics. Here again, however, there is virtually no discussion of chemical reactions in terms of molecular structure. The big book by Kauzmann is very largely devoted to atoms and the author never really crosses the border from physics into chemistry. Clearly, the word 'chemistry' means something quite different to these authors from what it means to the vast majority of chemists.

Now we have yet another book with the same title and a much braver effort. This new book, by Daudel, Lefebvre and Moser, represents a creditable attempt to set down in an orderly manner those developments which in recent years have made quantum chemistry a subject of real interest to the experimental worker. The centre of gravity of the book is the unsaturated hydrocarbons and their physical and chemical properties. This is not inappropriate, since until quite recently it would have been true to say that this particular field of study had yielded a greater crop of quantitative results than any other branch of theoretical chemistry. It is inevitable that a book of this sort should dwell on certain topics at the expense of others, and it would be easy to criticize the authors for having omitted any discussion of the recent striking advances in, for example, the theoretical chemistry of the transition elements. But this would be ungrateful, as the authors have obviously taken pains to include reference to the most important papers in the theory of conjugated molecules; the fact that their book is not as broad as its title suggests only bears witness to the great rate at which theoretical chemistry is now developing.

Turning to details, one is glad to have a clear account of the theory of antisymmetrized wave functions and the Hartree-Fock equations. Of less value is the short chapter on biochemical applications: we still know so little about the cell that it seems premature to attach any weight to a quantum mechanical theory of carcinogenesis. Again, the order of the chapters is perhaps a little odd; the two parts of the book are linked together by only a very tenuous thread, and it is strange to have a chapter on reaction-rates preceding one on chemical equilibria. Perhaps also in the second edition the authors might consider whether, for example, their long tables of bond angles are worthy of the space which they occupy in view of the very cursory discussion which is given to this set of data. But it is a fault on the right side to make too much reference to experimental results; it would indeed have helped the reader to appreciate the strength of the more sophisticated theory if more comparisons had been given between calculated and observed excitation energies in conjugated molecules.

Taken all in all, this book is certainly a useful addition to the literature.

H. C. Longuet-Higgins

## HIGH-PRESSURE TECHNOLOGY

### The Design and Construction of High Pressure Chemical Plant

By Harold Tongue. Second edition, revised. Pp. xii+250. (London: Chapman and Hall, Ltd., 1959.) 84*s*. net.

SINCE the first edition of this book was published in 1934 there has been a widespread increase in the use of high pressures in the chemical industry, particularly in the field of petrochemicals. New processes such as the polymerization of ethylene and the synthesis of diamond have been developed which operate at pressures and temperatures far greater than those attainable twenty-five years ago; furthermore, the advent of nuclear power has necessitated a great increase in the size of pressure plant. In order to describe the many developments in high-

pressure technology that have made this progress possible, the text has been added to and modified considerably, so that this edition may be regarded as a new book on the subject.

The chief aim of the author was to provide a practical guide to the design of industrial high-pressure plant; although the work is mainly concerned with equipment used in the chemical industry, the general background of high-pressure technology is broadened by frequent references to the techniques used in the design of nuclear power plant. The practical nature of the book is emphasized by the inclusion of an introductory chapter devoted to the various codes of practice, specifications and statutory requirements for the design and operation of pressure plant. It is unfortunate that the references to British Standard 1500 on "fusion welded pressure vessels for the chemical and allied industries" which occur throughout the book concern the provisional code issued in 1949 and not the extensively revised edition published in 1958.

Although the pressure vessel codes give detailed practical design procedures for vessels operating at pressures up to about 200 atmospheres, for higher pressures there are as yet no accepted codes and the chemical engineer must base his designs largely on first principles. It is with the principles governing the design, fabrication and choice of material of construction for highly stressed vessels that the first half of the book is concerned. The basic theory underlying the design of both monobloc and compound cylinders is supplemented by the detailed results of recent experimental work on the yield and rupture of thick-walled cylinders. In addition to the well-known methods of fabrication, such as hollow forging and fusion welding, the newer techniques of tape-winding and the multi-layer form of construction are described in detail. In view of the increasing use now being made of the autofrettage procedure for extending the elastic range and prolonging the fatigue life of thick-walled hollow cylinders, the chapter dealing with this subject is disappointing in that very little design information is given. The same criticism may also be made of the section concerned with the problems of creep at high temperatures; furthermore, no mention is made of the effect of fatigue on design. On the other hand, much useful information, not readily accessible in the literature, has been brought together in those sections of the book which deal with the low-temperature embrittlement of steels and with those corrosion problems peculiar to high-pressure plant.

The second part of the book, which is mainly concerned with ancillary equipment such as pumps, compressors and valves, provides the reader with well-illustrated and detailed descriptions of a large number of designs. Since the requirements of industrial processes are so diverse, the author has had to exercise careful judgment in selecting suitable examples to demonstrate the salient features of the various items of plant. In addition, much useful information is given concerning the techniques employed in a number of continuous high-pressure catalytic processes and the methods used for the preparation and purification of industrial gases.

Unhappily the author died earlier this year, soon after the publication of this edition of his first book. In compiling it, he has performed a valuable service to all those interested in the field of high-pressure technology.

K. E. Bett

# ANNUAL REVIEW OF ENTOMOLOGY

## Annual Review of Entomology

Vol. 5. Edited by Edward A. Steinhaus and Ray F. Smith. Pp. vii+451. (Palo Alto, Calif.: Annual Reviews, Inc., 1960. Published in co-operation with the Entomological Society of America.) 7 dollars.

THE aim of this "Annual Review" is to cover the whole science of entomology, pure and applied: and since the insects provide at least five-sixths of all known species of animals, are exceedingly diverse in form and function, have a tremendous influence on the economy and health of man, and are being studied by a very large army of investigators—there is no shortage of material for annual review. The study of some subjects has only recently begun. It is still possible for all work on the fine structure of insects to be summarized in a single article (G. A. Edwards). Cytogenetics of insects has been studied for many years and the review by S. G. Smith covers only selected highlights from the recent literature. A few years, ago a review dealing with the biochemistry or neurophysiology of insects would have been a rather unsophisticated affair, from sheer lack of knowledge. But that is no longer so; the article on insect flight muscles and their physiology (E. G. Boettiger) shows that in both these fields new basic ideas are being worked out on insects. In the physiology of neurosecretion, which is well reviewed by W. G. Van de Kloot the insects have always led the way.

The editors of the "Annual Reviews" maintain a constant effort to persuade their authors to present a point of view and not just an annotated catalogue of references. They score a fair proportion of successes with their entomologists. In writing on host selection in phytophagous insects A. J. Thorsteinson contests the assumption made in an earlier review by Fraenkel and Lippe that only the adventitious flavours of plants are utilized in host selection, never the nutrients themselves. But if a point of view is put too strongly a review can degenerate into a polemic. In surveying once more the question of population control, H. G. Andrewartha and L. C. Birch make a serious effort to get away from the sterile theoretical arguments about density-dependent factors and to discuss some actual contributions to the study of insect abundance in Nature. Mechanisms of resistance are again reviewed, this time by A. W. A. Brown, who quotes 229 references; a number exceeded only by L. R. Jeppson and G. E. Parman, writing on insects and mites as pests of citrus, who have 240. That represents about half of the twenty articles in this volume. The range of subjects covered is so wide that every year there is plenty of material to choose from which really needs reviewing. The high standard of earlier volumes is maintained this year.

The print in these volumes is rather small, which reduces the impact of their content on the reader; but it is pleasant not to have them printed on the highly sized paper so much favoured in the United States. However, a substantial number of pages in the copy received for review showed the letterpress on the other side of the page so clearly as to be difficult to read.

V. B. Wigglesworth

# THE LAND OF THE MARSUPIALS

## Biogeography and Ecology in Australia

Edited by A. Keast, R. L. Crocker and C. S. Christian. (Monographiae Biologicae, Vol. 8.) Pp. iv+640+18 plates. (The Hague: Dr. W. Junk, 1959.) 65 guilders.

THE continent of Australia is about the same size as the United States or 25 times the area of the British Isles. Sydney was settled in 1788, but it was not until about 1825 that other coastal areas began to be developed. The 'gold rushes' brought a great increase of population in the mid-nineteenth century. It was not until a little more than a century ago that the dawn of scholarship came with the establishment of the Universities of Sydney and Melbourne.

In the pioneer era, the studies of the infant departments of biology were of necessity concerned largely with the description of local fauna and flora. In later years, however, the pendulum of interest swung remarkably so that the chief studies of a majority of senior academic zoologists were of problems that could be rather more efficiently attacked in London or New York. The wonder, then, is not that Australians know so little about the ecology, biogeography and physiology of their native fauna, but (as is shown by the present volume) that they know so much. It is significant that much of the new zoological knowledge is not due to the efforts of university workers, but to officers of the splendid Commonwealth Scientific and Industrial Research Organization.

"Biogeography and Ecology in Australia" will be valuable not only for ecologists but also as a general reference book for university teachers, senior students and biologists in general. (For example, how many know that there are only 11 fewer eutherians than marsupials in "the land of marsupials"?) The book brings together much of the available knowledge, as well as enabling us to see where the gaps are. The first essay deals with "The Uniqueness of Australia in Biology" (by F. S. Bodenheimer) and then follow thirty-six chapters on the ecology of primitive aboriginal man, contemporary human ecology, that of the vegetation, the extant chordate classes, some of the major invertebrate groups and other topics. Pleasing evidence that, largely under the impetus of a Swiss and an Englishman, Australians are at last beginning to study the biology of Australian mammals is found in the chapters by Main *et al.* and Sharman. Among the hitherto unsuspected physiological adaptations of certain pouched mammals is rumination. Delayed implantation, too, has been demonstrated in two species by Sharman and Ealey and others within the past few years.

A. J. MARSHALL

# CLIMATIC ATLAS OF THE CZECHOSLOVAK REPUBLIC

## Atlas Podnebí Československé Republiky

Pp. 42+100 plates. (Praha: Ustřední Správa Geodesie a Kartografie, 1958.) Cena Kčs. 300.

THIS impressive book contains 89 charts of Czechoslovakia on the scale of 1 : $10^6$ and eleven diagrams. The maps, which measure about 2 ft. 6 in. by 1 ft. 6 in., are beautifully printed in attractive systems of different colours for areas of different values of the elements concerned. East and West Germany are the only other countries to have produced a climatic atlas with maps of so large a scale.

Detailed descriptions of the contents are given in English, Russian, and French as well as Czech.

The first nine charts give the administrative areas, river basins, soil types, forest regions and climatic regions based on Končeк's humidity index for the vegetation period.

Next there are thirty-one temperature charts which give mean monthly and annual isotherms of screen temperature (that is, not reduced to mean sea-level), mean numbers of days of maximum temperature over 25° C., of maximum below −0·1° C., etc., mean first and last frost days, and the mean duration of various periods such as the one of mean daily temperature 5° C. or more. The mean temperature of the day is defined as $(7)+(14)+2(21)/_4$, (7), denoting the temperature at seven hours, etc. In summer this probably gives a rather higher value than the customary British formula of average of maximum and minimum. As charts of mean values they are excellent, but for an area of such variable weather as Central Europe they much need to be supplemented by information on extremes. Extremes are not mentioned anywhere, yet other sources give the following table for Prague in January:

VALUES IN °F.

| Average daily max. | Average daily min. | Average highest in month | Average lowest in month | Absolute max. | Absolute min. |
|---|---|---|---|---|---|
| 34 | 25 | 45 | 8 | 55 | −16 |

The information outlined on average monthly extremes and absolute extremes is the least information on variability which ought to be included for scientific and economic purposes.

The section on precipitation contains mean monthly and annual amounts, means for the vegetation and winter periods and the mean numbers of days with precipitation exceeding 1 and 10 mm., days of snowfall and snow cover, etc. Next there is a section with maps of wind-roses, averages of relative humidity in July and December, mean cloudiness in September, November and the year, annual numbers of days of fog, sunshine, and thunderstorms. These are standard features of most climatic atlases, but for a country of mixed terrain like this much is lost by giving only annual values of sunshine since it is well known that there is a marked seasonal difference between the relative sunshine distribution in mountains and plains.

Finally, there are twelve phenological charts (mean date of apple-blossoming, harvesting oats, etc.) and eleven charts of seasonal variations of soil temperature. The latter, with examples for a hot summer and a severe winter, are the only sections of the book to give any indication of extreme values.

The period of the observations used is mainly 1901–1950 but is 1926–1950 for some and 1946–1953 for wind-roses. The preface mentions that it is intended to publish a volume of tables but suggests that this will contain only the data used in drawing the isopleths of the work under review. It is much to be hoped that the tables will in fact contain some data on extremes of temperature and precipitation if it is not possible to go all the way and provide an airmass climatology similar to that published in 1958 for West Germany (*Ber. deutschen Wetterdienstes* No. 45. "Zur Klimatologie der Grosswetterlagen", by K. Bürger.)

G. A. BULL

## Physics and Chemistry of the Earth

Vol. 3. Edited by Prof. L. H. Ahrens, Frank Press, Kalervo Rankama and S. K. Runcorn. (Progress Series.) Pp. viii+464. (London and New York: Pergamon Press, 1959.) 100*s*. net; 15 dollars.

THIS volume contains admirably concise chapters by J. W. Durham on palæoclimates, by A. G. W. Cameron on the origin of the elements and by D. C. Tozer on the electrical properties of the Earth's interior. S. I. Tomkeieff, in "Geochemistry in the U.S.S.R. (1954–1956)" gives a partially annotated bibliography of about 1,200 titles which is an extremely useful list of Russian publications dealing with igneous, metamorphic and sedimentary rocks, geochemistry, cosmochemistry, mineralogy and natural waters. It is a sad reflexion on the state of scientific libraries in Britain that some of these publications are unobtainable anywhere in this country. The geochemistry of thorium and uranium is reviewed by J. A. S. Adams *et al.*, while in "Silicate Melt Systems" E. Roedder reproduces about sixty phase-diagrams dealing with various portions of the geologically important system $K_2O$—$Na_2O$—CaO—FeO—MgO—$Fe_2O_3$—$Al_2O_3$—$SiO_2$, and he gives a helpful summary of the experimental techniques used. Unfortunately there is no attempt to relate the diagrams to petrology in the way pioneered by N. Bowen more than thirty years ago.

Nearly half the book deals with geophysical investigations in the eastern Caribbean by C. B. Officer *et al.* and the continental margin of the east coast of North America, north of Cape Hatteras, by C. L. Drake *et al.* Much undigested detail quite unsuitable for a general review is contained in both these articles, and this has probably been the chief cause of the increased price (100*s*.) compared with either Vol. 1 (70*s*.) or Vol. 2 (63*s*). For example, the former article contains no less than 55 pages of seismic profiles and their interpretation while the latter contains 13 pages on 'significant' boreholes in eastern America and the important basement rocks encountered ('decomposed rock'; 'bedrock'; 'crystalline rock'), various figures and text, all of which fail to give the reader a picture of the basement. B. E. Leake

## The Measurement of Grassland Productivity

Proceedings of the University of Nottingham Sixth Easter School in Agricultural Science, 1959. Edited by Prof. J. D. Ivins. Pp. viii+217. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1959.) 35*s*.; 7 dollars.

FOR several years the University of Nottingham has organized an Easter School in a branch of agricultural science. At the 1959 School, on measuring grassland productivity, 20 papers were given, which are published in this book, together with summaries of the discussions.

Grass occupies two-thirds of British farmland, but current production from it is much less than would be possible if existing knowledge of improved varieties, fertilizers and techniques of management were fully applied. Lack of confidence in methods for measuring grassland productivity hinders these developments and this School was organized to review and discuss current methods.

In the opening session Dr. William Davies reviewed the history of methods of measuring grassland output; corresponding German work during the past twenty years was described by Prof. E. Klapp. T. E. Williams then gave a good account of the effect of leys on the productivity of arable crops grown after the leys were ploughed.

A second group of papers dealt with the growth characteristics of different herbage crops and the problems of assessing their relative merits. Herbage has no true value until it has been used by an animal, so the third group of three papers discussed methods of assessing how much grass is actually eaten by stock in grazing experiments. Eight papers dealt with assessments of animal production from grass, and with correlating estimates of pasture growth with the performance of animals using the herbage. Three of these papers discussed the digestibility of herbage in pasture experiments.

Two papers described farm-scale measurements. Work done by Imperial Chemical Industries, Ltd., on commercial farms showed that measurements of grassland production must be related to output from the whole farm business; single measurements of productivity, and particularly physical measurements, can be misleading. Mr. Rex Patterson showed how his farm records of grazing have been used to measure pasture productivity and also quality.

The papers cover the subject well although their quality varies; together they provide a good review and the book is recommended to those concerned with scientific work on grass. G. W. Cooke

## Handbook of Toxicology

Vol. 3: Insecticides: a Compendium. By William O. Negherbon. Prepared under the Direction of the Committee on the Handbook of Biological Data, Division of Biology and Agriculture, National Academy of Sciences—National Research Council. Pp. xxv+854. (Philadelphia and London: W. B. Saunders Company, 1959.) 98*s*. net.

THE insecticides volume of the "Handbook of Toxicology" is an impressive production which is well calculated to save an immense amount of time for anyone seeking the available information about the great range of chemicals that are in use as insecticides and synergists at the present time. The contents are arranged alphabetically under the preferred chemical names of the substances. But interspersed among these are general articles on such subjects as anti-metabolites, bees and insecticides, cytochrome oxidase (effect of insecticides on), resistance, temperature and insecticidal action and others. Some of these sections help to lighten the subject-matter, as do the introductory sections dealing with each insecticide or group of insecticides. Under a given substance will be found an account of its chemistry; toxicity for man and wild-life; toxicity for insects; factors influencing toxicity; entry and mode of action in the insect; formulations; resistance; all these subjects being illustrated by exhaustive compilations of tabulated data. An insecticide such as DDT on which a vast literature exists will have about 40 pages devoted to it. Original references are quoted for every piece of information; the reference list contains 3,404 titles. There are separate indexes to chemical compounds, and to the scientific and common names of insects.

This should prove a most valuable work of reference. The short general introductions to the various sections can be read with interest by anyone looking for a broad survey of the subject.

V. B. Wigglesworth

# NUCLEAR TRAINING FACILITIES AT THE ROYAL NAVAL COLLEGE, GREENWICH

By Prof. J. EDWARDS

THE Royal Navy, with one nuclear submarine, *Dreadnought*, well under construction, and another likely to follow, with a land-based prototype plant at Dounreay also well advanced, and a possible interest in nuclear surface ships, has an obvious need to ensure a continued output of officers fully trained in the theory, techniques and problems of nuclear propulsion systems to meet its specific requirements. To provide this, the Department of Nuclear Science and Technology was set up under me at the Royal Naval College at Greenwich in January 1959.

The responsibilities of this Department were to provide courses suitable for those officers responsible for the operation of the nuclear submarines and for the prototype at Dounreay, and also to provide an advanced course suitable for those technical officers and civilians who would go to the Design Departments of the Admiralty concerned with nuclear propulsion. At present all reactors under construction for these purposes are of the pressurized-water type, and the courses are therefore aimed at this system in particular and the small mobile reactor system in general rather than the large gas-cooled graphite-moderated reactors used in the land-based power station programme.

The course for the submarine officers and operators lasts six months and that for the designers one year; both are of high academic standard demanding good mathematical knowledge, and cover atomic and nuclear physics, chemistry and metallurgy, reactor physics and kinetics, shielding and heat transfer, servo-mechanisms and control systems, and detailed information on the actual plant systems and components to be installed in the submarines. In the case of submarine officers of H.M.S. *Dreadnought*, they, on successfully completing the course, proceed to the United States to undergo a period of practical training in sea-going nuclear submarines of the United States Navy. This facility will later be replaced by training on Great Britain's own prototype at Dounreay when this is commissioned. Those officers and civilians who successfully complete the six-months course and who stay on for the advanced course spend a further term in which the main emphasis is given to a detailed design study of a mobile reactor system, to additional experimental work, and to a series of lectures from experts from the Atomic Energy Authority and industry related to safety aspects, metallurgical topics including radiation damage and fuel element fabrication, steam generator design and steam cycles, radiochemistry, advanced mobile reactor systems, and more detailed reactor physics and core design studies, relating to flux flattening, burn-up, control problems and the use of burnable poisons, etc. The design studies, although at first concentrating on the pressurized-water systems, will gradually be extended to cover other systems of interest to marine propulsion, namely, boiling-water reactors, organic moderated reactors, high-temperature gas-cooled reactor systems and others. These lectures are supplemented by additional visits to centres of nuclear research, fabrication and construction.

The six months reactor course is necessarily predominantly theoretical but nearly a dozen basic physics experiments have been introduced to give the students familiarity with the detection and measurement of radiation, and basic health and safety precautions and monitoring instruments. These cover the normal Geiger counter experiments, the measurement of half-value thickness of various materials, calibration of scintillation spectrometers, measurement of beta absorption curves, and the back-scattering of beta particles. Some neutron transmission experiments have also been introduced in which neutron cross-sections, as well as diffusion and slowing-down lengths of neutrons, are measured. The neutrons for these experiments are provided by a neutron 'howitzer', essentially a carefully designed storage shield for a polonium–beryllium neutron source of about 1 c. The neutron howitzer consists basically of a steel drum containing a 10-in. thickness of paraffin surrounding the source. A number of vertical and horizontal ports or beam holes are provided for the experiments together with means for accurately positioning the source and the experimental equipment.

An important facility from the point of view of the reactor operators is the reactor simulator. This has been built by Vickers-Armstrongs (Aircraft) Ltd., and is largely based on their *Mk. II* design modified in certain aspects to meet the specific requirements of the Department. These machines are based on analogue computer principles, and by means of electronic circuits the equations relating to neutron flux, reactivity and time in a nuclear reactor are continuously solved. Various configurations of fuel and moderator with an associated range of prompt neutron life-times can be represented, and studies of the effectiveness of automatic control systems and the changes of reactivity due to fission product poisoning, temperature coefficient and thermal transient effects can be carried out. The control panel instrumentation provides most of the important indications given on the control desk of a full-sized reactor, including pen recording on logarithmic and linear power scales, a logarithmic power-level meter, and a doubling time or period meter. Automatic start-up is provided, as well as a 'demand-power' function generator with meter display. A temperature effect is also built in, using a temperature proportional to power, with a suitable time-lag, a temperature trip and a meter display of average coolant temperature. Safety simulation is obtained by the incorporation of various safety trips and interlocks to provide actual reactor safety trip responses. A large range of experiments can be undertaken with the aid of this machine, and include reactor start-up procedure, approach to maximum power-level, step changes in reactivity, doubling time and reactivity variations,

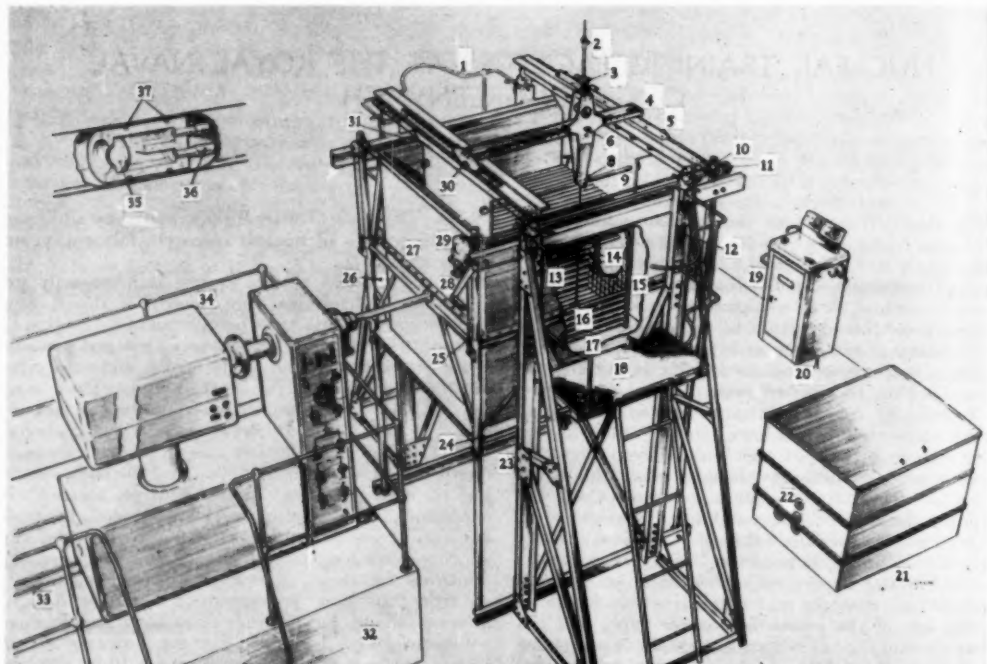

Fig. 1. Sub-critical reactor. Natural uranium light water moderated, pulsed neutron generator
1, handrail; 2, probe clamp; 3, probe adjuster; 4, indexed longitudinal beam; 5, indexed lateral angle; 6, ball lock; 7, probe locator; 8, probe guide wheels; 9, lattice plate spacer; 10, lateral movement hand wheel; 11, tank winch pulley; 12, jacking screw and hand wheel; 13, target (see detail); 14, alternative target holes; 15, lattice plate; 16, probe; 17, fuel elements; 18, operator's platform; 19, water purifier; 20, purifier drain; 21, small tank (unreflected system); 22, alternative target holes; 23, end frame assembly; 24, tank cradle locating peg; 25, tank winch cable tensioner; 26, tank cradle; 27, large tank; 28, winch handle and extractor; 29, tank winch gear-box; 30, ball lock; 31, lateral movement cable tensioner; 32, concrete plinth; 33, alignment rails; 34, particle accelerator. Details of target: 35, target; 36, cooling water pipes; 37, 'O'-type sealing rings

poisoning effects and simulation of 'accident conditions'. It therefore provides a comparatively inexpensive training device very suitable for experiments following on the theoretical teaching on reactor kinetics and control, and prior to actual power reactor operational training.

It is planned also to install shortly a sub-critical reactor in the Department (Fig. 1). This is being built by Miles-Hivolt and is largely based on a design evolved in the University of Birmingham, suitably modified to the Royal Naval College requirements to provide additional flexibility in the experiments to be carried out with this equipment. The facility is a light-water moderated, natural uranium assembly, using a small but powerful accelerator to produce neutrons. The latter can be used either as a continuous or pulsed source of mono-energetic neutrons, free of the $\gamma$-radiations emitted by most radioactive sources. It is easily transportable and can therefore be used independently for a variety of other nuclear physics experiments. The five tonnes of natural uranium rods are in the form of as-cast uranium rods canned in aluminium, 43¼ in. long by 1·22 in. diameter. These are supported in two vertical lattice plates to form a horizontal array on a square pitch of 1·9 in. to an accuracy of better than 0·005 in. The assembly is supported in a space frame at a variable height and contained within one of two stainless steel moderator tanks which can be winched into position and provide either an unreflected system with one tank, or some 6 in. of water reflector with the other tank. A dump tank, pump, and water treatment plant are also provided. A platform around the structure provides easy access for the assembly and positioning of monitoring and counting equipment, and a special traversing mechanism is supplied to enable accurate positioning of such equipment in three dimensions to be carried out. A source tube 1 in. in diameter passes horizontally through the system and is coupled to the 150-kV. ion accelerator. The deuterium or tritium target for neutron production can be placed at any position in the tube, which in turn can be placed in any one of four alternative positions through the lattice. The accelerator, which requires a power supply of about 8 kV.amp. and a cooling water supply of 5 gallons per min., is capable of giving a maximum beam current between 0·75 and 1·00 m.amp. either with hydrogen or deuterium ions and can be operated continuously, or with a pulse repetition rate between 0·65 and 3 kc./sec. giving a neutron output between $10^9$ and $10^{10}$ neutrons per sec. of either 3 or 14 MeV. energy, depending on the beam and type of target used. The advantages of using an accelerator as a neutron source instead of a

powerful radioactive source are that one no longer needs extreme care in the shielding, handling and transport of the source; one can literally turn the neutrons on and off at the turn of a switch, and no expensive and time-wasting re-irradiation of the source is required. Some of the experiments which will be carried out to give the students an understanding of reactor core phenomena and essential design parameters include measurement of buckling, multiplication factor, thermal utilization factor, criticality measurements, neutron migration lengths, prompt neutron reactivity, time constants of the assembly and neutron lifetime studies for prompt and delayed neutrons. The equipment, however, due primarily to the pulsed neutron facility, will also provide adequate opportunities for research to be undertaken by the staff of the Department.

Other experimental rigs now being designed include two heat-transfer loops, for gas and liquid heat-transfer studies, and a large-scale shielding experiment to be used in conjunction with the accelerator. To provide some additional experience with an actual critical facility short training visits are being arranged with Hawker-Siddeley's Nuclear Power Co. at Slough, to enable students to become acquainted with operational procedures and experimental uses of the firm's 10-kW. Jason reactor. It is hoped to obtain a more advanced computer than the reactor simulator mentioned above.

The first full reactor course of six months has now just been completed, and a second six-month course is about half-way through its final term, as is the first advanced course. Results have been most encouraging, and it is evident that a firm foundation for training in nuclear propulsion systems has now been established. Additional and more general courses in nuclear science and technology lasting two weeks and six weeks respectively are to be instituted in the near future to cater for those concerned with other and broader aspects of nuclear propulsion.

The Department is situated in one of the historic and beautiful buildings designed by Wren of the Royal Naval College, and although this posed some problems in its internal conversion to nuclear laboratories, the building has been adapted surprisingly well. Two laboratories are provided, one on the third floor and one on the ground floor, each with its associated offices and instrumentation test facilities. That on the third floor, which is in close proximity to the main offices, lecture rooms, stores and electronic workshop of the Department, is used for the basic nuclear physics experiments involving, in the main, sources of only low activity, generally less than 1 mc. Facilities are also provided here for preparation of specific sources from radioactive liquids. The other laboratory is designed to provide storage and handling space for sources of several curies of activity as well as facilities for the storage of any accumulated solid and liquid active waste. This laboratory will house the larger and heavier experimental equipment such as the sub-critical reactor, heat-transfer loops and the large-scale shielding experiments referred to above. Consideration has also been given to the possibility of ultimately housing a low-power research and training reactor. Clean room, offices and adequate crane lifting capacity have been provided together with a dark room for the processing of film used in some experiments and for instruction in radiographic techniques. The conversion of this laboratory is now practically complete and the installation and commissioning of the sub-critical facility in which the advance course students will participate will begin towards the end of September.

Fig. 1 is a drawing of the sub-critical facility.

I wish to acknowledge with thanks the permission of the Admiralty to publish this article.

# HUMAN AND OTHER ANIMAL CELLULOSE

By Dr. D. A. HALL and H. SAXL*

Department of Medicine, University of Leeds

PRIOR to the observations[1,2] that anisotropic fibres consisting partly of cellulose and partly of protein, exist as a minor but normal constituent of mammalian tissue, the only form of cellulose which could unequivocally be ascribed to the animal kingdom occurred in the tests of the tunicates. Evidence has also been advanced for the presence of cellulose in the outer layers of silk fibres from *Bombyx mori*[3], and other species of silkworm, but there is no evidence that this material is in any way oriented, nor is its identity proved beyond doubt[4].

We have therefore investigated the origin and comparative relationships of the cellulose-containing fibres of mammalian tissue. To do this, five different tissues have been examined, all rich in neutral polysaccharide, but differing markedly in site and function. These were: cotton fibres as representative of plant cellulose, anisotropic fibres from an affected area of the dermis of a case of scleredema adultorum of Buschke[5], a preparation from aged human aortic media, finely dispersed material from the basement membrane of pig kidney and the mid-portion of the test of the tunicate, *Ascidiella aspersa*.

## Polarization and Electron Diffraction Observations

Polarization and electron diffraction results were correlated. It has been observed previously[6,7] that tunicin is composed of layers of interwoven anisotropic fibrils, and in addition it contains anisotropic fibres or lamellæ similar in all respects to those of mammalian tissue. Frozen sections of human aortic tissue also show the presence of the mammalian type of anisotropic fibre resembling presumably the anisotropic structures reported by Hass[8] as early as 1943. Aortic tissue preparations were also obtained by the fractionation of the pooled media of aged human aortas (50–70 years old) after removal of the collagen by exhaustive extraction with boiling dilute acetic acid[9]. Fractionation with boiling 40 per cent urea

* Ida Smedley MacLean International Fellow of the International Federation of University Women.

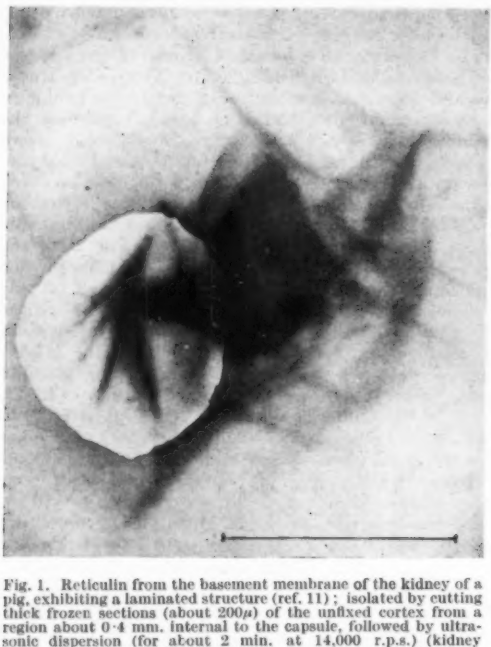

Fig. 1. Reticulin from the basement membrane of the kidney of a pig, exhibiting a laminated structure (ref. 11); isolated by cutting thick frozen sections (about 200$\mu$) of the unfixed cortex from a region about 0·4 mm. internal to the capsule, followed by ultrasonic dispersion (for about 2 min. at 14,000 r.p.s.) (kidney obtained from freshly killed animals). The light area is that from which the electron diffraction pattern, demonstrated in Fig. 2, was obtained (the method has been reported in ref. 2) ($\times$ 34,000)

solution gave, after dialysis and filtration, a solution of pseudoelastin[10] which also contained small anisotropic particles (0·05–0·1 mm. in diameter). Particles of about similar size and having the same optical properties were observed in suspensions containing reticulin from basement membranes of pig kidney obtained by ultrasonic dispersion (Figs. 1 and 2); although reticulin in its native state is normally regarded as isotropic[11], it would appear possible that the ultrasonic dispersion has revealed some material which has a marked degree of anisotropy. Dermis from the lesion of Buschke's scleredema contained large numbers of anisotropic fibres of mammalian type.

Although X-ray diffraction studies give a fibre diagram for plant cellulose, this was not observed with electron diffraction studies[2], since the small number of micelles gave a Debye-Scherrer diagram, which is characteristic of amorphous or polycrystalline material. A fibre diagram was, however, observed for scleredema and tunicate cellulose with electron diffraction methods, indicating a greater degree of orientation at this level of organization. Classification was, however, also possible on the basis of the relative intensities of the reflexions. Electron diffraction patterns of the aortic pseudoelastin[10] and renal reticulin preparations included reflexions which were indistinguishable from those of cellulose as exemplified by fibres from human dermal tissue, cotton or tunicin. The intensities of the various reflexions differed considerably however. The heights of the peaks in the densitometer tracings are recorded in arbitrary units chosen to suit each plate. No direct comparison can therefore be made between the absolute values in different tracings. Relative values are, however, comparable. The main observation which can be made from the two sets of curves (Figs. 3 and 4) is that the manifestly fibrous forms (plant cellulose and that from human pathological dermis) are characterized by reflexions of considerable intensity from the 021 and 002 layers. Haphazardly oriented material, on the other hand, shows a more general spread of intensities over a number of different reflexions. Tunicin, although fibrillar, demonstrates a diffraction pattern similar in many respects to that of pseudoelastin and reticulin, and it is thought possible that this similarity may be related to the presence of a layered structure. However, the diffraction pattern of tunicin is characterized by the fact that the reflexions form arcs, indicating the type of orientation which Mark and Susich[12] ascribed to layers aligned at a constant angle. Similar arcs were observed in patterns from the fibres of scleroderma tissue, where cellulose sheets surround the protein template in layers.

Not only can the various polysaccharides be identified as having similar electron optical properties by the positions of the various reflexions, but also the human fibrous form can be shown to react in exactly the same fashion as the plant material to partial hydrolysis with hydrochloric acid[13], which is accompanied by changes in the crystal structure characterized by alterations in the diffraction pattern. The most obvious change under the conditions employed here is an intensification of the third-order reflexion from the 021 layer relative to the adjacent reflexion from the 002 layer. As can be seen from Fig. 3, this change occurs equally in both human and plant cellulose.

## Biochemical, Histochemical and Electron Microscope Observations

These observations were made on the reticulin and the pseudoelastin fraction from aorta. Following intradermal injection of pectinase[14] into a guinea pig, microdissection of the reticulin layer of the dermal epidermal junction can be observed, and polysaccharide from this region is liberated into the tissue to form a broad band with positive periodic

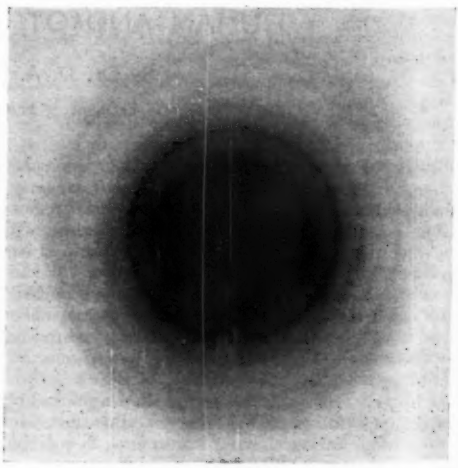

Fig. 2. Electron diffraction pattern from the area indicated in Fig. 1. The two most intense rings correspond to reflexions from the 101 and 021 layer lines of cellulose. The net plane indices of the other reflexions are indicated in the densitometer tracing in the upper section of Fig. 4. The broad diffuse band underlying the 021 ring represents the reflexion corresponding to the 2·86 Å. spacing of the haphazardly oriented array of collagen fibrils in the reticulin. Photographs taken with Elmiskop I

acid–Schiff reaction[15]. These observations could be related to the examination of bullous lesions of human dermis. *In vitro* studies demonstrated that the amount of polysaccharide liberated, following pectinase action, is much greater from sclerodermatous than from normal tissue. Electron microscope examination of the pathological dermis revealed how the formation of cellulose fibres from this polysaccharide might occur. It was suggested earlier[2] that the initial stage in the formation of anisotropic cellulose fibres in mammalian tissue may necessitate the presence of a protein template and that this might well consist of partially degraded collagen. In the case of reticulin, it appears that the template may in fact consist of a network of intact collagen fibrils. In pathological dermis, different stages in the transformation from reticulin-like fibrils to highly crystalline ones were observed. Cellulose micelles are preferentially adsorbed on to collagen in the regions of the $a$, $b_1$ and $b_2$ bands, thereby forming complexes which act as nuclei for the growth of cellulose crystallites.

High concentrations of neutral polysaccharide were also observed in the pseudoelastin preparations from arterial media. Total polysaccharide and uronic acid determinations made it possible to deduce that the neutral polysaccharide was firmly bound to a protein fraction, the so-called pseudoelastin which followed collagen but preceded elastin into solution in urea solution. This particular protein has the same amino-acid analysis as that reported for the protein fraction of anisotropic fibres in other tissues, and hydrolysis showed glucose to be the major sugar component. Not only was the solubility of old pseudoelastin (67 yr.) greater than that of a similar preparation from young tissue (20 yr.), but also the amount of cellulose-like neutral polysaccharide associated with it was increased. Apparently this was linked with an associated decrease in mucopolysaccharide content, since the total polysaccharide content was not greatly affected by age.

Frey Wyssling[16] has pointed out that interchain hydrogen bonds in the cellulose crystal lattice do not lie in the plane of the pyranose rings (the 002 plane) but in the diagonal 101, and to a less extent in 10$\bar{1}$ planes. The relative unimportance of the 002 spacing in preparations from reticulin, pseudoelastin and tunicin may therefore indicate that planar alignments of the cellulose chains are formed in these tissues, presumably due to the planar nature of the substrate on which they are deposited, as opposed to the three-dimensional crystal structure which results from deposition on a cylindrical template. Both reticulin and tunicin are known to be planar in arrangement, and it is to be expected that pseudoelastin, having been dried from solution, would crystallize in the simplest possible form. That such a simple structure is in fact in the form of a planar

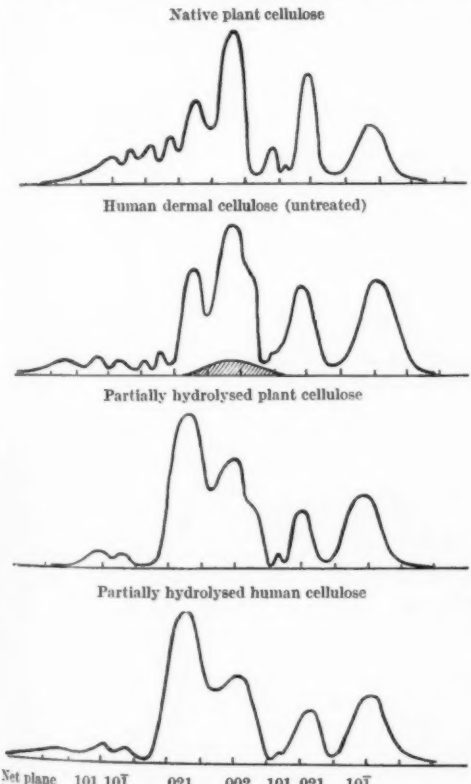


Fig. 3. Densitometer tracings of diffraction patterns from native plant and human cellulose (scleredema dermis) and from similar material after partial hydrolysis following 5 hr. boiling under reflux with an azeotropic aqueous hydrochloric acid solution

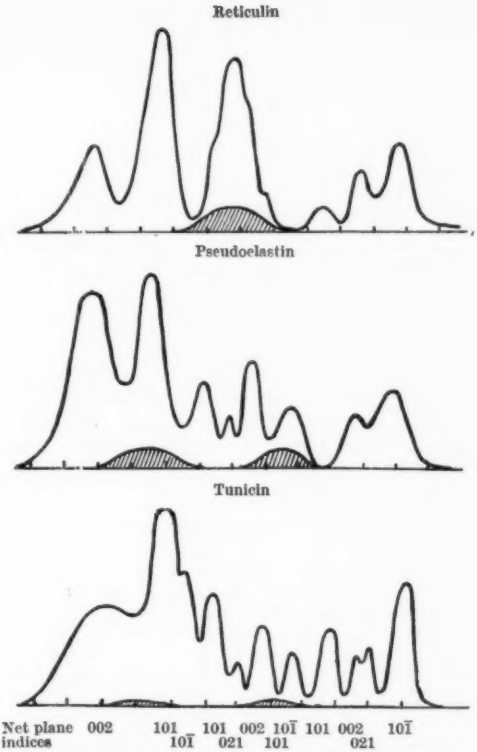


Fig. 4. Densitometer tracings of diffraction patterns from (*a*) pig kidney basement membrane (cf. Figs. 1 and 2); (*b*) a preparation (pseudoelastin) dissolved from human aortic media with boiling 40 per cent urea after the removal of collagen and ground substance with boiling 2 per cent acetic acid; and (*c*) a preparation of tunicin from the test of the tunicate *Ascidiella aspersa*. The shaded areas refer in (*a*) to the diffuse reflexions from the 2·86 Å. spacing in collagen, and in (*b*) and (*c*) to reflexions characteristic of the 2·0 and 4·4 Å. spacings of elastin

array has been demonstrated by Mukherjee, Sikorski and Woods[17] for mechanically or chemically dispersed plant cellulose microfibrils. It would appear therefore that, in the presence of a suitable substrate, hydrogen-bond formation in the 101 plane completely supercedes any interchain reactions in other planes, with the consequent formation of a layered cellulose structure. In all these cases, however, the presence of reflexions of low but finite intensity from the 002 plane must indicate that at least a portion of the polysaccharide has become associated with a template on which it is capable of forming a three-dimensional array and hence of producing a fibril. This deduction is in complete agreement with the observations of a limited number of anisotropic structures in these preparations.

The apparent similarity between the different protein polysaccharide complexes would appear to be of considerable significance. In all cases examined, the protein consists of either collagen or a degradation product of this, pseudoelastin. The nature of pseudoelastin has been discussed before[10]. The present work demonstrates that not only does pseudoelastin contain a basic amino-acid pattern but it also is a protein polysaccharide complex. Only in the case of tunicin and the mammalian dermal fibres has the polysaccharide been unequivocally identified as cellulose. The pseudoelastin fractions, by the nature of their method of preparation, cannot be identified as single compounds, but evidence from electron diffraction diagrams, examination in polarized light and chromatographic assessment of the sugar content of their acid hydrolysates would appear to indicate that the polysaccharide resembles cellulose, at least in so far as it consists in the main of β(D)-glucose molecules joined through 1 : 4 linkages. However, since similar anisotropic structures to those proved to contain cellulose can be observed in fibres containing chondroitin sulphate[3], it may not be necessary for all the linkages between the sugar residues to be 1 : 4.

That tunicates may represent the ancestors of the vertebrates has been discussed by several workers[18,19], and the similarity between the protein fractions to which mammalian and tunicate celluloses are attached may represent further evidence in support of this hypothesis. It does not appear inconceivable from the present observations that the polysaccharide–protein complex of reticulin may have been developed phylogenetically from the ancestral neutral polysaccharide–protein complex tunicin, on the pathway from tunicate to man, since only quantitative biochemical and biophysical changes would be required for such a development. Pontecorvo[20] has considered the possibility that evidence is available at a structural rather than a metabolic level for evolutionary development to be due either to changes in, or maintenance of, specific genetical characteristics. The appearance of increasing amounts of cellulose-like polysaccharide in combination with protein in ageing aortic tissue may indicate that similar genetical changes may occur during the ageing of an individual.

A fuller discussion of these results and their implications will be given elsewhere.

This work was mainly carried out while we were members of the Nuffield Gerontological Research Unit, University of Leeds. We thank Prof. R. E. Tunbridge, Dr. F. Happey and Prof. R. D. Preston for much critical discussion, and Prof. H. Magnus for the specimen of dermis from a case of scleredema, and Dr. H. J. Woods for densitometer facilities.

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# COMPARISON OF FISSION PRODUCT AND BERYLLIUM-7 CONCENTRATIONS IN THE ATMOSPHERE

By W. ANDERSON, R. E. BENTLEY, R. P. PARKER, J. O. CROOKALL and L. K. BURTON

Physics Department, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3

SCINTILLATION spectrometry provides a convenient method of following the levels of specific γ-ray-emitting nuclides, and, since May 1959, we have been measuring the γ-activity of atmospheric dust collected from surface air by an electrostatic precipitator at Sutton, Surrey[1]. Apart from one sample which contained material from the first French nuclear test[2], we have been unable to measure the relatively short-lived zirconium-95 plus niobium-95 in samples collected after December 1959. We have therefore considered the principal γ-emitting fission products of longer half-life[3], shown in Table 1. The 0·51 MeV. γ-line of rhodium-106 was thought to be particularly suitable for following current trends in concentrations of fall-out materials in the air. However, difficulties are encountered due to the presence of other nuclides with similar γ-energies.

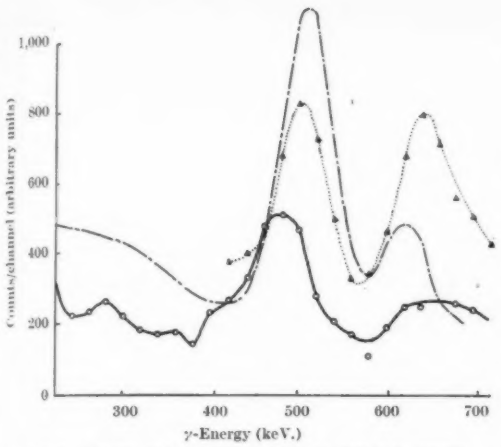


Fig. 1. Spectra of dust samples obtained with 3-in. crystal. ---, May 1959; ——, December 1959; —·—·—, rhodium-106 reference standard

Fig. 1 shows the γ-spectra of two typical dust samples, one collected during May 1959 and the other during December 1959, measured with a 3 in. × 3 in. sodium iodide (thallium) crystal and 100-channel analyser. The spectrum of a rhodium-106 reference standard is also shown and it is found that the position of the principal peak in the spectrum of the May sample differs by not more than half a channel width from that of the standard. One channel width of our spectrometer corresponds to an energy difference of 0 020 MeV. In samples collected after December 1959 small but significant changes in the position of this photopeak were observed. Thus the position of the principal peak in the spectrum of the December sample is about 1·5 channels width from that of the standard, indicating an energy about 0·03 MeV. lower than the accepted value of 0·51 MeV. for rhodium-106[4]. This peak decayed initially with a half-life of about 70 days.

## Detailed Analysis of 0·5 MeV. Peak

In order to determine more accurately the γ-energies contributing to the peak in the region of 0·5 MeV., the γ-spectra of several samples were measured using a 1-in. cube sodium iodide (thallium) crystal which has a lower efficiency than the 3-in. crystal previously employed but an improved energy resolution (8 per cent at 0·66 MeV.). Part of the spectrum of our mid-February 1960 sample is shown in Fig. 2 and it will be seen that the peak at about 0·5 MeV. is complex. One component appears to be at an energy slightly higher than 0·50 MeV. This is probably due to rhodium-106 since in addition we have observed a small peak in the spectrum at 0·62 MeV. (Table 1). If it is assumed that rhodium-106 is present, the intensity of the 0·62 MeV. line can be used to estimate the contribution of the 0·51 MeV. line to the composite peak, since the relative intensity of these two lines is known from the reference standard. The composite peak can then be resolved into three components with maxima at about 0·515 MeV., 0·476 ± 0·005 MeV. and about 0·43 MeV. Each of these components was found experimentally to have the same resolution as a single photo-peak of corresponding energy, which suggests that there are only three main components. For the reasons given below, the 0·476 MeV. line has been attributed to beryllium-7 and the 0·43 MeV. line to antimony-125.

## Identification of Antimony-125

Antimony-125 is known to occur as a fission product[3], and has been reported in soil samples taken near the site of a nuclear explosion[5]. Its possible occurrence in ryegrass has also been suggested[6].

Apart from the correspondence of the 0·43 MeV. peak (Fig. 2) with the energy of the principal γ-line of antimony-125, we have detected an X-ray line of 0·027 MeV. which would be expected from the tellurium-125m daughter of antimony-125. The additional γ-lines of antimony-125 at 0·60 MeV. and 0·17 MeV. were masked by γ-lines from other fission products (barium-137m, rhodium-106 and cerium-144).

Evidence for the chemical identity of the nuclide responsible for the 0·43 MeV. γ-line was provided by addition of antimony carrier to a dust sample, subsequent separation of the antimony fraction and examination of this by γ-spectrometry. It may be noted that the material thought to be antimony-125 remained associated with the insoluble fraction after evaporation of the sample with a series of concentrated and dilute acids (perchloric, hydrochloric, hydrofluoric and nitric). This marked insolubility suggests that the activity was incorporated in highly inert, probably fused, particles. The insoluble matter was fused at 600°–650° C. with a mixture of equal parts of potassium hydroxide, potassium carbonate and potassium nitrate, and with the addition of 10 mgm. each of silver, antimony, cerium and strontium as carriers. The melt was leached with water and the lixiviate was found to contain the activity suspected of being antimony-125. After acidification with hydrochloric acid, the Group 1 precipitate was centrifuged out and the antimony recovered from the supernatant as the sulphide. The γ-spectrum (Fig. 3) of this antimony sulphide was identical with that of an antimony-125 reference source.

## Identification of Beryllium-7

Beryllium-7 is known to occur naturally[7], being produced in the atmosphere by the interaction of

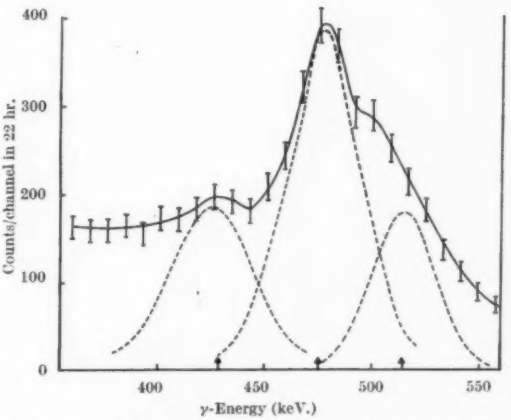


Fig. 2. Detailed analysis of 0·5 MeV. peak obtained with 1-in. crystal

Table 1. Nuclear Data and Atmospheric Concentrations in Surface Air of Non-gaseous Nuclides

| Nuclides | Half-life of parent | Principal γ-lines | | Maximum β-energy, MeV., principal mode of decay | Concentration in air February 1960 | |
|---|---|---|---|---|---|---|
| | | Energy MeV. | No. of transitions per 100 disintegrations of parent | | c./litre | atoms/litre |
| Fission products | | | | | | |
| $^{106}Ru \rightarrow {}^{106}Rh$ | 370 days | | | 0·04 | $4 \times 10^{-18}$ | 7 |
| | | 0·513 | 21 | 3·5 | | |
| | | 0·621 | 10 | | | |
| $^{125}Sb$ | 2·4 yr. | 0·175 | 6 | 0·3 | $2·5 \times 10^{-18}$ | 10 |
| | | 0·427 | 29 | | | |
| $\rightarrow {}^{125m}Te$ | | 0·595 | 32 | | | |
| | | 0·027* | 20 | | | |
| $^{137}Cs$ | 30 yr. | | | 0·5 | $\sim 10^{-16}$ | $\sim 50$ |
| $\rightarrow {}^{137m}Ba$ | | 0·662 | 83 | | | |
| $^{144}Ce$ | 285 days | 0·134 | 8 | 0·3 | $7 \times 10^{-18}$ | 9 |
| $\rightarrow {}^{144}Pr$ | | | | 3·0 | | |
| $^{95}Zr$ | 65 days | 0·723 | 49 | 0·4 | less than $10^{-18}$ | < 0·3 |
| | | 0·756 | 49 | | | |
| $\rightarrow {}^{95}Nb$ | | 0·765 | 100 | 0·2 | | |
| Cosmic-ray produced nuclides | | | | | | |
| $^{7}Be$ | 53 days | 0·478 | 11 | No. β's, 100 per cent electron capture | $2 \times 10^{-17}$ | 5 |
| $^{22}Na$ | 2·6 yr. | 1·28 | 100 | 0·5 ($\beta^+$) | $10^{-20}$ | 0·04 |
| $^{32}P$ | 14 days | — | — | 1·7 | $5 \times 10^{-19}$ | 0·03 |
| $^{33}P$ | 24 days | — | — | 0·25 | $2 \times 10^{-19}$ | 0·02 |
| $^{35}S$ | 87 days | — | — | 0·17 | $10^{-19}$ | 0·4 |

* X-ray line.

cosmic ray nucleons with nitrogen[8]. The energy of the main photopeak in the spectrum shown in Fig. 2 was found experimentally to correspond very closely with that of a beryllium-7 standard. However, a search of nuclear data tables[4] showed three other possible nuclides with principal γ-emissions in the energy-range of 0·46–0·49 MeV. and half-life between 10 and 100 days, namely tellurium-129m, hafnium-181, and curium-241. In view of the short half-lives (35–53 days) of all four nuclides it is unlikely that they would have been detectable if they had been products of nuclear explosions. It is improbable that beryllium-7 is produced in such explosions[9]. Moreover, the fact that beryllium-7 is continually produced in the atmosphere makes it the most probable of these four nuclides as being responsible for the 0·476 MeV. peak. The absence of additional γ- and X-ray energies which we would probably have detected from any of the other three nuclides adds weight to this view.

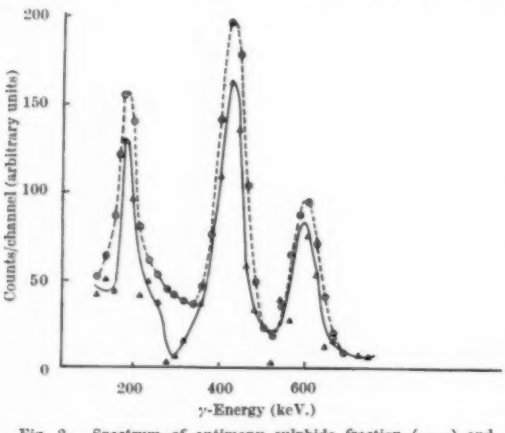


Fig. 3. Spectrum of antimony sulphide fraction (——) and reference standard of antimony-125 (- - - -)

We now turn to the chemical evidence for the identity of this material. An appropriate dust sample was digested with 50 per cent hydrochloric acid containing 10 mgm. of beryllium as carrier, and the 0·476 MeV. γ-line was found in the spectrum of the extract. The ready solubility of the activity is in marked contrast to that of antimony discussed above and accords with that to be expected from the mode of formation of beryllium-7, presumably as widely separated atoms. The solution was treated with an excess of ammonia to precipitate beryllium hydroxide and other hydroxides derived from elements such as aluminium and iron normally present in atmospheric dust[10]. The mixed hydroxides were extracted with 2·5 *N* sodium hydroxide to dissolve the beryllium and aluminium, leaving various hydroxides, notably that of iron, as an insoluble residue. The alkaline extract was next scavenged with hafnium by acidification, addition of hafnyl chloride and precipitation of hafnium hydroxide with excess alkali. Antimony and tellurium were removed together from the alkaline beryllium solution by successive acidification, addition of tellurium carrier, and precipitation of the sulphide. The beryllium was recovered from solution by precipitation with excess ammonia. A further scavenge, analagous to that with hafnium, was carried out using cerium. The hydroxide scavenges should have removed any curium that might be present.

In order to obtain the beryllium as a small source for β- and γ-counting, and to provide further purification, it was next separated from the associated aluminium. The supernatant from the cerium hydroxide precipitation was just acidified with hydrochloric acid and oxine reagent added, followed by ammonium acetate to complete precipitation of the aluminium. After removal of the aluminium oxinate by centri-

Table 2. Ratio of Specific Activities of Fission Products, at a time 18 months after Fission, calculated from Fission Yield Data for Different Types of Fission

| | $\frac{^{95}Zr+^{95}Nb}{^{144}Ce}$ | $\frac{^{106}Rh}{^{144}Ce}$ | $\frac{^{125}Sb}{^{144}Ce}$ | $\frac{^{137}Cs}{^{144}Ce}$ | $\frac{^{90}Sr}{^{137}Cs}$ |
|---|---|---|---|---|---|
| $^{233}U$ $n_{th}$ | 0·15 | 0·07 | 0·004 | 0·11 | 0·93 |
| $^{235}U$ $nf$ | 0·17 | 0·10 | 0·02 | 0·13 | 0·71 |
| $^{239}Pu$ $nf$ | 0·17 | 1·7 | 0·06 | 0·17 | 0·36 |
| $^{238}U$ $nf$ | 0·15 | 0·60 | 0·08 | 0·15 | 0·48 |
| $^{235}U$ $n_{14}$ | 0·17 | 0·95 | 0·24 | 0·17 | 0·51 |
| $^{233}U$ $n_{14}$ | 0·19 | 0·43 | 0·32 | 0·16 | 0·82 |
| Experimental value Feb. 1960 | <0·14 | 0·57 | 0·36 | ~0·14 | — |

$n_{th}$, thermal neutrons; $nf$, fission spectrum neutrons; $n_{14}$, 14 MeV. neutrons.

fugation, the beryllium was precipitated with excess ammonia and mounted on a planchette. The $\gamma$-spectrum of this precipitate consisted solely of a line at 0·476 MeV., and was identical with that of a beryllium-7 reference standard. There was no measurable $\beta$-activity.

## Relative Concentrations of Fission Products

Table 1 gives our observed concentrations in air of fission products associated with atmospheric dust during February 1960. Table 2 gives the relative activities of a number of nuclides eighteen months after fission, computed from published data[3] for different fissile materials and various neutron energies. It should be noted, however, that these ratios may need modification when applied in the case of a nuclear detonation. It will be seen that the ratios rhodium-106 : cerium-144 and antimony-125 : cerium-144 are sensitive to the type of fission process. Taken together, our experimental ratios (Table 2) agree best with the theoretical values for 14 MeV. fission of either uranium-235 or uranium-238, and in particular the high antimony concentrations which we have observed do not appear compatible with data for thermal neutron fission of uranium-235. The 14-MeV. neutrons are unlikely to be produced in a normal fission process, but they could be the result of a fusion reaction.

## Concentration of Nuclides produced by Cosmic-rays Relative to Fission Products

Table 1 includes data for non-gaseous cosmic-ray produced nuclides. With the exception of beryllium-7, for which our measurements are quoted, the values are estimated from published concentrations in rainwater[11] on the assumption that the relative amounts in air and in rain are the same as for beryllium-7. It should be noted that the activity of beryllium-7 measured by us lies within the range of values 0·5–15 $\times$ $10^{-17}$ c./litre of air (1–30 atoms per litre) reported in Canada in 1956[12].

Table 3 contains some preliminary measurements of the specific activities of beryllium-7, rhodium-106, and antimony-125 in air samples collected during the early months of 1960. The seasonal rise in the beryllium-7 concentration is well known[12], and it is interesting to note that the fission-product concentrations are changing proportionally. This is demonstrated by the constancy of the ratios of antimony-125 : beryllium-7 and rhodium-106 : beryllium-7 activities.

Table 3. Rhodium-106, Antimony-125 and Beryllium-7 Concentrations in Air Samples

| Mean collection date | Specific activity at time of collection in $10^{-17}$ c./litre | | | $\frac{^{106}Rh}{^{7}Be}$ | $\frac{^{125}Sb}{^{7}Be}$ |
|---|---|---|---|---|---|
| | $^{7}Be$ | $^{106}Rh$ | $^{125}Sb$ | | |
| 2/1/60 | 1·4 | 0·26 | 0·12 | 0·19 | 0·09 |
| 30/1/60 | 1·6 | 0·24 | 0·18 | 0·15 | 0·11 |
| 18/2/60 | 2·2 | 0·41 | 0·27 | 0·19 | 0·12 |
| 4/3/60 | 3·4 | 0·48 | 0·32 | 0·14 | 0·09 |

It is noteworthy that by the beginning of 1960 the specific activity of fission products in the air at ground-level has fallen to a value comparable with that of naturally produced beryllium-7. The presence of beryllium-7 and antimony-125 complicates the estimation of the absolute amount of rhodium-106.

We wish to express our appreciation of the guidance and encouragement of Prof. W. V. Mayneord, in whose laboratories the work was carried out. We also wish to thank Dr. D. M. Taylor for help in preparing reference standards.


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# OBITUARIES

## Dr. O. J. Silberrad

The thinning ranks of those British chemists who were trained in Germany before the First World War were further diminished on June 17 by the death of Dr. Oswald John Silberrad at his home at Dryads' Hall, Loughton, Essex, at the age of eighty-two.

Silberrad was educated at Dean Close Memorial School, Cheltenham, the City and Guilds Technical College and the University of Würzburg. In 1902, at the early age of twenty-four, he became the first superintendent of the Chemical Research Department at Woolwich, which was under the ægis of the War Office. The new Department could claim to be the first to be created on modern lines specifically for armament research, although the Government organization that is now the Chemical Inspectorate had from its inception, half a century before, concerned itself with research into materials and explosives as well as with its primary function of inspection, and there was also a military experimental establishment concerned with ballistics.

The next four years were busy ones for the young superintendent. He went to Germany to study the comparable organizations there and designed the new laboratories and other equipment. He gathered a small but enthusiastic staff around him, including as his chief assistant and deputy Dr. R. C. Farmer, a fellow student at Würzburg who, with Dr. Godfrey Rotter (later director of explosives research in the establishment) and Dr. Harold Moore (later director, British Non-Ferrous Metals Research Association), among the seniors, survives him.

This period saw not only the discovery of tetryl and of the means of causing lyddite to detonate effectively in shell (it had conspicuously failed to do this in the Boer War), but also the introduction of T.N.T. into the Service and research into flashless and smokeless propellants and into steels for guns, shells and armour.

Silberrad reported to the Explosives Committee of the War Office, the chairman of which was Lord Rayleigh. In 1906, this Committee was disbanded, and in the same year Silberrad resigned, to become for the rest of his career a consulting research chemist and director of the Silberrad Research Laboratories. Had he remained in Government service, where he was such a distinguished pioneer of modern 'conventional' armaments research, there is no doubt that high office and honours could have been his.

His connexion with the armaments field was not, however, completely severed by his resignation, for in 1908 he was instrumental in providing the Admiralty with a special alloy capable of resisting the erosion suffered by the propellers of high-speed turbine-driven warships. Because of this erosion, the future of such vessels and liners had appeared somewhat problematical. Also, in conjunction with the firm of Hotchkiss, he worked on erosion-resisting steel for guns. Then, in the First World War he was honorary consulting chemist to the Ministry of Munitions. An outstanding achievement of this period was the successful process for manufacturing large charges of lyddite in iron instead of earthenware vessels. This enabled an improved synthesis, requiring a higher temperature than was practicable with the original method, to be used. He also turned his attention once more to flashless propellants and developed one for use in large howitzers.

Silberrad was a brilliant experimenter, and among other achievements of his fertile brain were a new chlorinating agent; a method of manufacturing isoprene; a new means of blasting petroleum wells; and the production of dyestuffs from T.N.T. residues.

I have very pleasant memories of a visit paid to Dryads' Hall and its adjoining laboratory in 1953, when I was collecting material for an article in connexion with the golden jubilee of Silberrad's old establishment, which by then had become the Armament Research Establishment of the Ministry of Supply. I was most hospitably and kindly received by Dr. and Mrs. Silberrad and given access to many old photographs and exhibits, over the assembly of which much trouble had been taken. This first, and unfortunately the last, personal contact with Dr. Silberrad left an impression of a man of intense mental vigour, drive and alertness, in spite of his seventy-five years and the fact that at the time he had not fully recovered from an attack of thrombosis.

Mrs. Silberrad survives her husband, with the only son of the marriage.

J. S. Grew

## Dr. Eric Ellenbogen

Dr. Ellenbogen was born in Vienna in 1921 and emigrated to the United States in 1940. He received his B.S. degree in chemistry from Indiana University in 1943. During 1943–46 he served with the United States Army in India and Burma. After his discharge, he studied at the Brooklyn Polytechnic Institute, working on inorganic plastics. In 1946 he entered Harvard University, received his M.A. in 1947 and his Ph.D. in 1949 under the guidance of Dr. J. L. Oncley. His dissertation dealt with the determination of the physical-chemical properties of insulin. From 1947 until 1949 he was a teaching assistant in the University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University. He was awarded a U.S. Public Health Service Postdoctorate Research Fellowship in 1949, which he held until 1951 in the Department of Biochemistry, Columbia University College of Physicians and Surgeons, under the sponsorship of the late Dr. E. Brand, studying the properties of tri- and tetrapeptides of specific steric configurations. In 1951 he was appointed research associate in the Department of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh, and was promoted to assistant professor in 1952 and associate professor in 1957.

In 1959 he took leave from his post at the University of Pittsburgh to travel to the Weizmann Institute at Rehovot, Israel, to study with Prof. E. Katchalski, doing research on physical properties of certain polypeptides as models for certain proteins of biological significance. On the way back from the Weizmann Institute to the United States, Dr. Ellenbogen died of a coronary occlusion in Marseilles on May 29, 1960. He is survived by his wife Lois and two children.

He belonged to several American and British learned societies. His contributions to biochemistry include the study of the physical-chemical behaviour of insulin in dilute solution, the physical chemistry of specific polypeptides, the isolation of a non-insulin hypoglycæmic factor from pancreas, and the physical-chemical characterization of cardiac myosin.

Robert E. Olson

## Prof. Eduard Reichenow

Eduard Reichenow, the eminent German protozoologist, died on March 23 in his seventy-seventh year. He was born in Berlin and was the product of a race versed in the natural sciences: his father, Anton, was a well-known ornithologist; his grandfather, Cabanis, a zoologist of note. He himself married Lilly Mudrow, of international fame in the field of malariology and who collaborated with him in his later work on avian malaria; her death three years ago cast a cloud over his life from which he was scarcely able to emerge.

Reichenow was educated at Schiller-Realgimnasium in Charlottenburg; later he entered the Universities of Heidelberg, Berlin and Munich, where he graduated in natural science.

He was associated with the Institute of Tropical Medicine, Hamburg, for most of his academic years, first as a student, for many years as director of its Department of Protozoology, and finally as its president. He travelled extensively in the pursuit of his work, and some of these journeys produced results of great scientific value—the discovery of malaria parasites in gorillas in the Cameroons, the

elucidation of the complicated life-cycle of *Karyolysus* and other hæmogregarines in Spain, and the development of piroplasms in animals in East Africa. He investigated Chagas's disease in Venezuela, Mexico and Guatemala, sleeping sickness in Africa, marine biology at Rovigno and in the Baltic. In Germany itself he studied the intestinal protozoa of man, and with his wife he described in detail exoerythrocytic schizogony in *Plasmodium relictum* and other avian species of malaria parasites.

His careful observations on the cycle of piroplasms in the tick led him to conclude that this important group of protozoa belonged more probably to the Sarcodina than to the Sporozoa, among which they had hitherto been placed, and this new view may well act as a guide to their still unknown nature.

Reichenow's work and reputation were consolidated in his successive editions of Doflein's "Protozoology". Doflein's widow asked him to undertake this task and he produced the fifth edition in 1929; the sixth was not completed until 1952. The history of the latter provides a good illustration of his strength of character—the manuscript of the new edition was practically finished during the later years of the Second World War when a bomb fell on his house and the entire work was destroyed; undeterred, he recommenced the task and completed it a few years later.

Eduard Reichenow was a simple, quiet man, cautious and conservative in his scientific outlook, with an absence of flamboyance which did not detract from his ability to disentangle complicated problems in taxonomy or biology. His sound approach made him an ideal court of appeal, and he readily responded to requests for his opinion from all quarters of the globe. In these respects he resembled the English protozoologist, the late C. M. Wenyon, whose views on many problems coincided with his own.

He received many honours towards the end of his life: he was made an honorary member of the Society of Protozoologists, given an honorary doctorate of the University of Granada, and received special recognition from Munich on the occasion of the jubilee of his graduation.

The death of Reichenow has left a gap in the ranks of protozoologists which it will take long, if ever, to fill. He is survived by his daughter.

P. C. C. GARNHAM

# NEWS and VIEWS

## Physics at University College, London: Prof. J. Hamilton

DR. J. HAMILTON has been appointed to the newly created second chair of physics in University College, London, from October 1. Dr. Hamilton studied in the Queen's University, Belfast, and later began research at the Dublin Institute for Advanced Study under Heitler. During the War he was engaged on scientific work for the Royal Navy. After a brief appointment as lecturer in theoretical physics at the University of Manchester he moved to Cambridge in 1950 as lecturer in mathematics and was elected to a fellowship at Christ's College. Dr. Hamilton's interests have always lain on the physical side of mathematical physics, and during his time at Cambridge there has been a considerable strengthening in the amount of physics available to students reading the Mathematical Tripos. He has also succeeded in forming an active group of young workers imbued with his own pragmatic spirit. Under his leadership they have been applying modern dispersion relation techniques to low-energy pion physics with most interesting results. This characteristic emphasis on physical content is also to be found in his recent book, "The Theory of Elementary Particles", which contains valuable chapters on phenomenology as well as those dealing with the development of quantum field theory. The appointment of Dr. Hamilton to University College will strengthen considerably the theoretical side of a department which is already active in experimental high-energy nuclear physics.

## Mechanical Engineering at Cardiff: Prof. S. P. Hutton

DR. S. P. HUTTON, who has been appointed to the new chair of mechanical engineering at the University College of South Wales and Monmouthshire, was educated at Calday Grange Grammar School and the University of Liverpool, where he graduated in 1941. After a period in industry, he joined the Aerodynamics Department of the Royal Aircraft Establishment in 1942 and was concerned with the design, development and use of high-speed wind tunnels for research on sub-sonic and trans-sonic aircraft. During 1945–46 he was in charge of similar research facilities in Germany. During 1946–49 he was a lecturer in fluid mechanics at the Imperial College of Science and Technology, where he was awarded his Ph.D. for research on water turbines and fans. Since 1949 he has been with the National Engineering Laboratory, East Kilbride, where he supervised the design and commissioning of the hydraulic machinery laboratory, eventually becoming head of the Fluid Mechanics Division in 1954. During 1951–52 he was sent by the Department of Scientific and Industrial Research to the Federal College of Technology, Zurich, where he carried out research under Profs. J. Ackeret and H. Gerber. Since March 1959 he has been deputy director of the National Engineering Laboratory responsible for industrial liaison. His research interests are in engineering fluid mechanics and his publications have been mainly concerned with flow measurement, fans, pumps and water turbines. He is a member of the Hydraulics Group Committee of the Institution of Mechanical Engineers and has been closely associated with the work of national and international standards organizations for water turbines. He has travelled widely and in 1956 was a member of the British Hydroelectric delegation to the U.S.S.R.

## Chemical Technology at Edinburgh: Prof. P. H. Calderbank

DR. P. H. CALDERBANK has been appointed to the chair of chemical technology in the University of Edinburgh in succession to Prof. K. G. Denbigh, who is going to the Imperial College of Science and Technology, London (*Nature*, January 2, 1960,

p. 14). The chair will be held concurrently with the headship of the Chemical Engineering Department of the Heriot-Watt College. Dr. Calderbank, who is forty-one years of age, was educated at Palmer's School, Essex, and at King's College, London, where he graduated in 1940 with first-class honours in chemistry. He then joined the Ministry of Supply (Explosives Division), where he was concerned with the maintenance and operation of heavy chemicals plant. Afterwards, he joined the firm of Bakelite, Ltd., in Birmingham, and engaged in several research and development projects connected with the manufacture of synthetic resins and polymers. In 1946, Dr. Calderbank was appointed lecturer in the Chemical Engineering Department at University College, London. At University College, Dr. Calderbank undertook research on the kinetics of reactions in fluidized beds of catalysts and was awarded the degree of Ph.D. In 1952, he was appointed professor of chemical engineering in the University of Toronto, where he was an active member of the American Institute of Chemical Engineers. He joined the Department of Scientific and Industrial Research in 1955, and, after a four-month study tour of American universities and research establishments, returned to the National Chemical Laboratory at Teddington, where he proceeded to form and direct a research group of chemical engineers. This research group was transferred to the Warren Spring Laboratory at Stevenage in 1958, and under Dr. Calderbank's direction evolved into a team of forty workers engaged in many aspects of chemical engineering and process development research. Dr. Calderbank is a member of the Institution of Chemical Engineers and has been the recipient of the John Millar Medal for chemistry and the senior Moulton Medal for chemical engineering.

## Atomic Energy Research Establishment Appointments

Dr. F. A. Vick, at present deputy director of the Atomic Energy Research Establishment, Harwell, has been appointed director. Dr. R. Spence, at present chief chemist, has been appointed deputy director, and Mr. L. Grainger, now head of the Metallurgy Division, has been appointed assistant director, with special responsibilities for applied research. The new appointments will take effect on September 1. Sir Basil Schonland continues as director of the Research Group, of which the Atomic Energy Research Establishment is a part.

## Overseas Civil Service
### Supplementary Pay and Allowances

In statements made simultaneously in the House of Commons and in the House of Lords on July 28, it was announced that, subject to Parliamentary approval of the expenditure involved, the Government has decided to offer to take over, where necessary in Colonial territories, the inducement pay and allowances of overseas staff broadly on the lines of the arrangements followed in most technical assistance schemes. Although financial arrangements have already been reached with Nigeria and Sierra Leone in the light of their coming independence, it is also proposed to offer them agreements on those lines and it is intended to apply the arrangements to the High Commission territories. The effect of these proposals would be that, while the local Government would continue to bear the local rates of salary and other conditions of service, the British Government would bear the cost of supplementary pay and allowances which prove to be needed for overseas officers. The statement also recognized the direct responsibility of the Government to maintain stable administration throughout the years of change preceding self-government, while pressing forward with the recruitment and training of officers from the local population. These new arrangements, which will not cease with independence, demonstrate the importance which the Government still attaches to the maintenance of an efficient Overseas Civil Service. They would apply to all its 15,000 members, and the cost is estimated by the Secretary of State for the Colonies, Mr. Iain Macleod, at £10–20 million a year over ten years.

## Agricultural and Scientific Research Overseas

In reply to a question in the House of Lords on July 28, about agricultural and scientific research overseas, Lord Hailsham stated that both the Agricultural Research Council and the Department of Scientific and Industrial Research, like the Medical Research Council, have powers to maintain research workers in overseas territories where this is in the national interest, and the Department of Scientific and Industrial Research has some research staff in stations overseas. Both the Department and the Agricultural Research Council assist overseas territories by seconding staff for definite periods within the limits of their resources, and proposals for extending these arrangements are under examination. While it is considered that existing powers should be adequate for any such extension, Lord Hailsham said that the Government would not hesitate to seek additional powers if necessary.

## India's First Agricultural University

The Government of India has approved the establishment of an Agricultural University, the first of its kind to be planned in the country, at Rudrapur, in Uttar Pradesh. The University, a product of Indo–American co-operation, will be set up on the model of land grant colleges in the United States and is expected to cost Rs. 1.90 crores. The 16,000-acre Tarai State Farm will be attached to the University for use as a laboratory for research and experiment. The University will have three constituent colleges, an Agricultural College, a College of Animal Husbandry and Veterinary Science, and a College of Agricultural Engineering and Technology, and will train 325 students each year. It will provide comprehensive education in agriculture and allied subjects on the basis of an integrated system of teaching, and will pay particular attention to developing rural leadership and competence. The decision to establish the Agricultural University is to be welcomed, particularly at the present juncture, when considerable emphasis is being laid on self-sufficiency in food in the third Five-Year Plan.

## Dental Research

The Minister for Science, Lord Hailsham, stated in the House of Lords on July 27 that current annual expenditure on dental research of the Medical Research Council exceeds £35,000, and a considerable proportion of this sum is used to support investigations into the early development of dental caries at

all ages, including the influence of biochemical and bacteriological factors, and the role of fluorine, calcium and other elements in tooth formation and resistance to decay. This expenditure amounts to about 1 per cent of the Council's total expenditure on medical research. He understood that the value of hand-produced sugar confectionery, including chocolate confectionery, in 1959, was £193 million and that the total consumption, based on prices paid by consumers, was £259 million. There is also some £750,000 a year spent on dental work in the Universities through the University Grants Committee, but he agreed that even taking into account work done in private institutions, the total effort falls far short of the country's needs. Lord Hailsham pointed out that one limiting factor is the availability of suitable research workers, and that the school dental service, betterment of which could do much to improve the dental health of the nation, is already handicapped by the shortage of dentists.

## Output of Power in Great Britain

In his presidential address to the British Electrical Power Convention on May 30, Viscount Chandos, discussing "Electricity—the New Horizon", emphasized the great problems with which the increase in the capacity of generators faces the electricity manufacturers. The five main manufacturers of heavy-power plant in Great Britain had a capacity of not less than 7,000 MW. a year and the probable demand for new additions to plant for the grid might be put at about 2,750 MW. a year, leaving 4,250 MW. for export. The competitive power of Britain must be largely governed by competitive wages, and if these plants had to work appreciably below 7,000 MW. a year the industry would feel the pinch. To some extent, he pointed out, the amount of power that Britain has to sell for export must depend on the development of power in what are now described as under-developed countries, and in this connexion, while noting the general shortage of capital, Lord Chandos protested against the tendency to lend to uncredit-worthy countries. Besides avoiding inflation, Britain should seek to create new credit to match the ever-increasing sources of wealth which science and technology had to offer. Besides the new horizons in the generation of electricity, there were new horizons in its application. Its use in the home, the mounting use of fractional horse-power motors, its use in lighting and in communications, whether by transport or telephone, offered possibilities which it was as yet difficult to discern precisely, quite apart from the effect of such developments on all aspects of living. Finally, referring to the fact that the European Common Market would lead to some large industrial amalgamations, which would have at hand a larger domestic market than could ever be hoped to be found in Britain or even in the European Free Trade Area, Lord Chandos emphasized the danger of wasting or misusing technical man-power, instancing the waste involved in submitting four tenders for one nuclear power station. Without undermining competition, he suggested attempts should be made to co-operate more in fundamental research over a wide field and the industry should not be open to the charge of having half a dozen different research establishments, using perhaps twice the necessary technical effort, to extend the frontiers of our knowledge in relation to one particular part of the electrical industry.

## The Swedish National Committee for Physics

The tenth general physics conference of the Swedish National Committee for Physics was held at the Royal Institute of Technology in Stockholm during June 1–3, 1959. In addition to a large number of contributed papers presented at twenty sessions, special guest lectures were delivered by H. Maier-Leibnitz (Munich), who spoke about the research programme of the Munich reactor, and by C. J. Gorter (Kamerlingh Onnes Laboratory, Leyden), who discussed the problem of orienting atomic nuclei at low temperatures. Introductory papers were given by O. Klein on the systematics of elementary particles and by H. Alfvén on plasma physics and magnetohydrodynamics. Abstracts of all the contributed papers are published in the March issue of *Arkiv för Physik* (**16**, 481 ; 1960). Most of the papers deal with aspects of plasma and nuclear physics, but several are concerned with the infra-red examination of semiconductors. A number of new instruments and devices are reported. At the University of Uppsala a high-precision curved quartz crystal gamma spectrometer of the Du Mond type has been constructed and used for precision measurements of the *K*- and *L*-levels in the heavier elements. Optical instruments include shearing interferometers for the examination of spectral line broadening (Royal Institute of Technology, Stockholm) and a photo-electric device for measuring spectrograms (University of Lund). At the Nobel Institute of Physics, Stockholm, a new method for measuring half-lives of excited states in the region $< 10^{-10}$ sec., based on the high-frequency deflexion of conversion electrons, has been tested. As a first step in a programme of development of large bubble chambers to be used at the 1,200-MeV. electron-synchrotron at the University of Lund, a 2-in. cylindrical chamber with vertical windows has been constructed. This University has also produced a cylindrical dose-rate meter for X-rays which is of small dimensions (2-mm. diameter) and which utilizes a $p$–$n$ junction in a gallium arsenide semiconductor. The meter has a relatively low impedance and fast response, which makes it well suited for control measurements during X-ray therapy.

## Plankton at Freetown, Sierra Leone

In work on the plankton of the estuary and the immediate offshore waters of the Sierra Leone River (Colonial Office. Fishery Publications, No. 13. Pp. iv+48. London : H.M. Stationery Office, 1960. 35*s*. net) Mr. V. Bainbridge was faced with a two-part problem imposed by the alternation of wet and dry seasons, the resulting conditions differing very considerably. Throughout the wet season there is a tendency towards a two-layered transport system, with brackish water flowing seawards on the surface and more saline water flowing into the estuary along the bottom. These conditions are, however, scarcely detectable in the dry season. During the wet season most of the phytoplankton within the estuary consisted of benthic forms and, at the offshore station, diatoms were relatively scarce. Very small numbers of diatoms at the offshore station during the dry season were coincident with a large standing crop of phytoplankton within the estuary, comparable in abundance with those found in Long Island Sound and the Rio de Vigo. The zooplankton species appeared to be subjected to a flushing out from the

estuary during the wet season, with only a limited transport of a few species back into the estuary in the bottom current. Offshore, the largest variety of zooplankton species occurred late during the dry season, and fluctuations in species composition and abundance seemed to be associated with the oceanic currents. The very heavy standing crop of phytoplankton in the dry season did not appear to be utilized to anything like the full extent possible, either by zooplankton or by the phytoplankton-feeding clupeid fish *Ethmalosa dorsalis*. This fish, locally named the 'bonga', supports an important fishery in the estuary. The thorough examination of the sequence of events in this tropical estuarine area offers a very useful model for the approach to many similar problems, particularly in other areas where a fishery for *Ethmalosa* may be of local importance.

## 'Telex' and Inter-Library Communications

To arouse interest in the use of 'Telex' in inter-library communication a sub-committee of the Reference, Special and Information Section of the Library Association has compiled a directory listing 131 libraries in the United Kingdom with access to 'Telex' (Pp. ii+15. London: Library Association, 1960). The list was compiled on the basis of a questionnaire sent to the 198 members of the Association of Special Libraries and Information Bureaux, the firms of which are listed in the United Kingdom Telex Directory for October 1958, and to a few other organizations which had notified their interest in response to a notice in Aslib Proceedings of October 1959. The Directory is not for sale, but copies have been sent to public and special libraries, and it is hoped that the libraries listed will come to accept that 'Telex' can play a much greater part in library and information work in future, and that many more libraries will now consider it worth while to instal 'Telex'.

## U.S. Translations of Russian Biological Publications

The American Institute of Biological Sciences announces that it is now translating and publishing seven Russian research journals in biology. These translations are produced with support from the National Science Foundation. Hence the Institute is able to offer these translations at a fraction of their publication cost, with further price reduction to members of the Institute and to academic and non-profit libraries. The journals currently being translated are: *Doklady (Biological Sciences Section)*; *Doklady (Botanical Sciences Section)*; *Doklady (Biochemistry Section)*; *Plant Physiology*; *Microbiology*; *Soviet Soil Science*; and *Entomological Review*. In addition to the translations of Russian biological journals, the Institute has instituted a separate programme of translation and publication of selected Russian monographs in biology. The monographs that have been published are: "Origins of Angiospermous Plants", by A. L. Takhtajan; "Problems in the Classification of Antagonists of Actinomycetes", by G. F. Gauze; "Marine Biology", Trudi Institute of Oceanology, Vol. 20, edited by B. N. Nikitin; "Arachnoidea", by A. A. Zakhvatkin; and "Arachnida", by B. I. Pomerantzev. Further information can be obtained from the American Institute of Biological Sciences, 2000 P Street, N.W., Washington 6, D.C.

## Analytical Biochemistry

The aim of *Analytical Biochemistry* (published by Academic Press, Inc., 1, No. 1, 1960; 15 dollars) is to publish work on new and improved methods of preparation, purification and analysis of substances which are of interest to biochemists, biophysicists, and others in related fields of experimental biology. It will include contributions dealing with qualitative as well as quantitative methods of analysis, and with the design of special apparatus and instruments. The first number (June 1960; pp. 95) contains, among others, communications on flame photometry, scintillation counting, separation of serum lipoproteins, and column analysis of nucleic acids. The editor is Alvin Nason, McCollum-Pratt Institute, Johns Hopkins University, Baltimore.

## The Calouste Gulbenkian Foundation, Lisbon

The trustees of the Calouste Gulbenkian Foundation, Lisbon, have announced the grants made during the first half of 1960. They include grants of up to £1,000 per annum for three years to British undergraduate scientific expeditions, given in consultation with the Royal Geographical Society, as follows: £250 to the Cambridge expedition to British Guiana; £250 towards the Oxford expedition to the Peruvian Andes; £140 to the Oxford Women's expedition to the Azores; £100 to the University of Oxford expedition to Cyrenaica; and £50 to the Wye College Exploration Society expedition to Morocco. Grants in aid of educational institutions include £10,000 to the Oxford Institute of Education and £2,700–£3,400 over two years to the Institute of Education, University of Durham. The grants made in aid of education in Great Britain and the Commonwealth total £23,085. In Portugal, £109,257 has been granted in aid of science and medicine, including the establishment of a centre for the treatment of alcoholism and addiction at the Institute of Psychiatry; campaigns against tuberculosis in Portuguese Guinea, and malaria in the Island of Santiago, Cape Verde Islands: a total of £23,278 has been given to educational institutions. £9,000 has been given for scholarships for Iraqi technicians to study at the National Laboratory of Civil Engineering, Lisbon. Grants have also been allocated to institutions in Brazil, France, Chile and Iran.

## Abominable Snowmen

The problem of the 'Abominable Snowmen' is discussed by S. V. Obruchev (*Priroda*, **10**, 60; 1959), who presents a critical review of articles and books on this subject, beginning with Waddell's report of 1898 about the hairy wild man called 'Yeti' by the Tibetans. In the Soviet Press during 1957–59 there appeared a number of articles on this subject, especially on the possible presence of the 'Snowman' in the Pamirs. In 1958 a special expedition sent to the Pamirs to study this problem reported negatively, saying that the 'Snowman' existed only in local legends. Similar legends existed also in Mongolia. At the same time, numerous recent finds of teeth of a huge anthropoid ape—*Gigantopithecus*—in China suggest that the Tibetan 'Yeti' and the legendary 'Snowman' of the Pamirs and Mongolia may all refer to the former, or present-day but rare, presence of this type of ape in the high-altitude regions of the Himalayas and the Pamirs.

## U.S.S.R. Fisheries Research by Submarine

VALUABLE oceanographic and fisheries work has been accomplished by a specially constructed submarine, *Severyanka*, as described by V. P. Zaitzev (*Priroda*, **8**, 71; 1959). The submarine is provided with a viewing apparatus, several projectors, mechanical arms for collecting specimens from the bottom of the sea, echo-sounding apparatus and a cinema apparatus. The first sailing in the Barents Sea took place on December 14, 1958. Nine scientists were aboard. The second expedition took place during December 29, 1958–January 21, 1959, in the northern part of the Atlantic Ocean, and which covered some 4,000 nautical miles, aimed at the study of herring movements.

## National Science Foundation Awards for Graduate Research Facilities

THE U.S. National Science Foundation has awarded grants totalling 2,153,710 dollars to fifty-four colleges and universities to improve graduate research laboratories. These grants are made on condition that the institution concerned provides at least 50 per cent of the funds needed. This permits assistance to a larger number of financially hard-pressed institutions and at the same time stimulates self-help to the greatest extent possible by the institution. Grants provide for construction, conversion or modernization of laboratory facilities.

## The Institute of Metals: Annual Autumn Meeting

THE fifty-second annual autumn meeting of the Institute of Metals will take place at Bath during September 5–9. The meeting will be held partly at the Pump Room and partly at Guildhall. Included in the programme are such topics as: "Engineering Properties of Nickel-Base Alloys" and "Lubricants for Cold Rolling Aluminium" (September 5); "Recovery and Recrystallization" and "Engineering Properties of Alloys" (September 6); "The Relative Merits of Extrusion and Other Methods for the Manufacture of Bar, Rod and Wire-Rod" and "Magnesium Alloys for Fuel–Element Components" (September 8). The Autumn Lecture, on "Metals and the World of Engineering Structures", will be given by Sir Alfred Pugsley on September 9. Further information can be obtained from the Secretary, Institute of Metals, 17 Belgrave Square, London, S.W.1.

## Announcements

DURING the past few years, many entomologists have inquired for insect suction traps which segregate the catch by dropping disks into the collecting tube. One firm may now agree to make the 9-in. Vent-Axia type (as described in *Annals of Applied Biology*, **37**, 80; **38**, 582) for approximately £100 each, complete with time switch, provided fifty orders are placed. Will those interested please communicate with Dr. C. G. Johnson, Rothamsted Experimental Station, Harpenden.

A STEERING Committee, originally appointed as a working party by the Faraday Society to consider the future of its work in the field of biophysics, has decided to launch a new and independent society to be known as the British Biophysical Society. The chairman and secretary of the Steering Committee are Prof. J. A. V. Butler and Dr. J. C. Kendrew respectively. The first scientific meeting of the new Society will take place in London in December. A more detailed announcement, giving information about the new Society and about its first meeting, and inviting inquiries from prospective members, will be made shortly.

SUPPLEMENTS 9 and 10 to the Index-Catalogue of Medical and Veterinary Zoology issued by the United States Department of Agriculture, June 1959 and April 1960, respectively, covering authors from A to Z, are now available (Supplement 9. Pp. 1+322. 1.25 dollars. Supplement 10. Pp. i+357. 1.25 dollars. Washington, D.C.: Government Printing Office, 1959 and 1960).

THE fifth annual conference of the British Society for the Philosophy of Science will be held at Wills Hall, Bristol, during September 23–25. Further information can be obtained from Dr. M. B. Hesse, Whipple Museum, Free School Lane, Cambridge.

AN International Congress on Experimental Mechanics will be held during November 1–3, 1961, in New York City. Further information can be obtained from Dr. Roscoe Guernsey, jun., General Engineering Laboratory, General Electric Company, Schenectady 5, New York.

THE twenty-first National Conference for the Preservation of the Countryside will be held at Weymouth during October 13–15. Further information can be obtained from the General Secretary, The Council for the Preservation of Rural England, 4 Hobart Place, London, S.W.1.

THE Metallurgy Society of France is organizing a meeting on "The Heat Treatment of Metals and Alloys" during October 17–21. The meeting will be held at la Maison de la Chimie, 28 bis, rue Saint-Dominique, Paris 7e. Further information can be obtained from the Secretary-General of the Society, 25 Rue de Clichy, Paris 9e.

THE tenth annual Instrument Symposium and Research Equipment Exhibit are to be held at the National Institutes of Health, Washington, D.C., during October 3–6 and October 4–7, respectively. Further information can be obtained from V. B. Davis, National Institutes of Health, Public Health Service, Bethesda 14, Maryland.

THE second International Plastics Exhibition will be held in Utrecht during October 19–26. Immediately preceding the Exhibition, an international congress on "The Technology of Plastics Processing" will be held in Amsterdam during October 17–19. The congress is sponsored by the Association for the Advancement of the Knowledge of Materials, the Royal Institute of Engineers, the Royal Netherlands Chemical Federation and the International Union of Pure and Applied Chemistry. Further information can be obtained from N. V. 't Raedthuys, Terselschadestraat 5, Amsterdam.

ERRATUM. In the communication entitled "Synergetic Action of 2(*o*-Methoxyphenoxymethyl)-5-Oxazolidone on Morphine, Dihydrocodeine and Aminopyrine Analgesia", by E. Fischer, J. L. J. Szabó and M. Stamburgo, in *Nature* of June 11, p. 893, the authors state that they gave the chemical name of metoxadone incorrectly; it should read 5(*o*-methoxyphenoxymethyl)-2-oxazolidone in both title and first line of text.

# GRADUATE SCHOOLS IN BRITISH UNIVERSITIES

A FEW weeks ago some highly interesting findings were published* of a Committee set up at Oxford last November "to consider and report on the accommodation, facilities and amenities in the University for graduates of this and other universities". The facts unearthed and the suggestions made show the fast-growing importance of Oxford as a centre of graduate study, particularly in the sciences, and the urgency of catering for the needs of this graduate population in new ways. Clearly, there is among Oxford dons a body of informed opinion conscious of practicable measures which might be taken to help to deal with the situation. But the report of the Committee has wider implications, too.

In 1939, out of some 5,000 students at Oxford, 520 —or about 10 per cent—were reading for advanced degrees or diplomas; by 1960 this number had grown to 1,758 out of nearly 9,000, or about 20 per cent. Among the contributory factors to this development are the greatly increased numbers of grants for graduate work, the spread of the practice that scientists should take a research degree as a prelude to an industrial as well as an academic career, and the growing number of graduates from other universities who want to work at Oxford. Not merely have the modern universities much more than twice the student population that they had twenty years ago, but also Oxford and Cambridge have an unrivalled attractiveness both as places of learning and as prestige institutions ("above the social strife"). For many a graduate they have, consciously or unconsciously, something of the appeal of an Athenæum Club as well as of a university, short though they are of bedrooms and somewhat deficient in comforts. But so far neither has quite caught up with itself as a centre of graduate migration. Traditionally, they have been places at which to get a good undergraduate education, in a college, between the ages of eighteen and twenty-one or so. To-day, however, 92 per cent of Oxford's graduate students are more than twenty-two years of age, and a quarter of them more than twenty-five; 56 per cent come from other universities and are strangers to Oxford. Many are married, and of these the majority have their wives with them; an appreciable number have their children as well.

What is being done to meet the problems brought about by this state of affairs? The colleges provide some living accommodation, and a certain amount of wise advice is given about lodgings by the University Delegacy. But some of the lodgings are up to fifteen miles from Oxford itself and many stipulate "no children". The costs of good or even satisfactory accommodation are considerable; and outside the city there is much competition for it from Harwell and the surrounding air bases. The Committee believes that colleges ought to extend to unmarried graduates from other universities the privilege of spending at least their first year in college; and that the University ought to be bold in building flats for married couples and in making grants or loans to colleges to help them to convert houses for the use both of single and of married students.

* *Oxford University Gazette*, Supp. to Issue No. 3048 (June 16, 1960).

But the graduate may find a roof to cover him and still remain a lonely man, with remarkably little to make him feel that he really belongs not merely to a laboratory or a department but also to the University—and this at a time when it may be of great importance to him to feel that he does so belong. He may, it is true, be a member of his college junior common room and go to lunch at Halifax House (424 of the 544 members of which, incidentally, are science graduates), but Halifax House suffers from inadequate buildings and from having nothing like enough money. (The Committee notes that "it is hoped that a new building for Halifax House will be included in the major building programme for 1964–68". One would have expected a note of greater urgency than this.) That graduate common rooms should be provided in the colleges is an admirable suggestion (only one college at present has such a room); so is the idea that 'study rooms' should be made available for the use of married graduates living out. It would be a great help, too, if the Delegacy of Lodgings made itself an active centre for relaying information affecting the residence outside college of members of the University. In the wealth of their information services, their skilfully compiled maps and brochures, their welcoming spirit efficiently incorporated into administrative terms, American universities certainly have something to teach both older and newer universities in Britain.

It has to be remembered that many of the men and women going from modern universities to Oxford and Cambridge as graduates will not have been resident before, either at school or in a university. This is a new start for them, an introduction to a wider world than they may have hitherto experienced. There may have been little possibility of finding in their honours school at 'Redbrick' the diverse and yet friendly community which a good college can be: and their time at 'Oxbridge'—in a fresh environment and with widening horizons—may find them ready to be newly and intimately receptive. But the defect of the British social system generally—and one which on occasion has applied to graduates going up to Oxford and Cambridge, whether from 'Redbrick' or from overseas—is that it leaves so many feeling that they are not quite within the inner circle. This defect is one which the Committee certainly had at the back of its mind. Its suggestions are essentially suggestions about how to make Oxford a more potent educational force in the lives of the graduates studying within it.

The report, however, has a more wide-spreading significance than for Oxford or 'Oxbridge'. Discernible here is a shift in attitude to the whole problem of the welfare and education of the graduate student and a recognition that if his continued studies are to give him not merely more knowledge and a further degree but also continued—and general—education he must be considered in his own right. The development of graduate schools of character and power is not dependent on the provision of laboratories, libraries, research amenities and supervision alone. This, so far, we have tended not sufficiently to acknowledge: all British universities

remain unduly orientated to undergraduate needs. Yet the proportion of graduates will rise everywhere during the coming ten, twenty, thirty years; at some universities indeed (at the Imperial College of Science and Technology, London, for example) this policy is already fast being implemented.

In a period when it is the done thing to marry and have children early, especially in the professional classes, and when a parental means test for the graduate student must before long become a thing of the past, is enough thought being given even in the modern universities to the problems involved? The idea of raising the percentage of graduates accommodated in halls of residence by a variety of means, including the addition of specially planned halls for graduates, must be considered. At some universities already a few houses have been admirably adapted to yield numbers of flats for graduate students (though sometimes at the risk of isolating the graduate further from the life of the university); and there are several active graduate students' councils. But the special needs and aptitudes of the graduate for a liberal education as well as for research training have been little regarded.

No doubt, Oxford and Cambridge are likely for some years to come to be magnets to graduates from elsewhere. But other universities, too, are attracting increasing numbers of students from outside themselves (including some from Oxford and Cambridge) and it is surely good that this should be so. Experience of two universities of differing temper and situation is often likely to be more enriching than of one only.

This pioneer report from Oxford is much to be welcomed—for its encouraging facing of local facts; for its wealth of practical ideas; but also for the example it gives other universities to set in motion corresponding investigations and to think thoughts of their own on their provision for the graduate within them.

W. R. NIBLETT

# THE INDIAN OCEAN EXPEDITION

THE Special Committee on Oceanic Research, meeting in Copenhagen and Helsinki in July, was set up by the International Council of Scientific Unions in 1957, on the grounds that a new international scientific organization was needed to strengthen and promote research in marine science. It was to initiate joint programmes in consultation with the scientific unions and other organizations. The Committee met for the first time in the Woods Hole Oceanographic Institution in August 1958, and among other measures proposed a joint expedition to the Indian Ocean. It tried to choose the area most likely to attract the attention of scientists, where the work would appeal to world organizations which promote science and human welfare, and where better understanding is likely to prove most immediately useful to the surrounding countries. The Indian Ocean was an obvious choice[1].

The complete change-round of some of its currents in response to changes in wind direction will give physicists a fairly unique opportunity to study factors that influence water movements. Better knowledge of accompanying biological changes will improve our quantitative understanding of factors that control oceanic productivity. More detailed information about the distribution of fish—especially those that live in mid-ocean—in relation to the boundaries between currents and water layers should advance fisheries science and be readily usable. The Indian Ocean is an ocean with exceptional and prominent features. The exchange of energy between sea and air has much to do with the powerful monsoon winds, and any improvement in long-term forecasting of their onset and intensity will be very useful. We know there are large fish populations in mid-ocean, and records of tremendous mass mortalities occurring from time to time show that they live in critical marginal conditions. In geophysics, as in other aspects, the Indian Ocean offers attractive sites for research.

The justice of trying to boost science by pledging the anticipated success of a new scheme has rightly been questioned, and there is no doubt of the sincerity and good intentions of the editor of the privately circulated "Indian Ocean Bubble", which has voiced very reasonable opposition. He does not quite say that skilled young scientists who form the real backbone of our research laboratories will be swindled by the promoters, but he criticizes the prospectus which probably has promised too much, and sounds a clear warning about the ill effects of hindering new approaches and critical experiments by too much regimented and routine work. He makes chilling reference to recent books, "The Tragic History of the Sea", by C. R. Boxer — three accounts of disastrous early Indian Ocean expeditions—and "The South Sea Bubble", by John Carswell—a general popular account of the venture that he frightens us with.

The effort devoted to marine science is, however, in no way commensurate with the magnitude, variety and difficulty of the problems it has to solve, and after a hundred years work, much of it devoted to fishery problems, we have little idea about the distribution of current with depth in any ocean. We can quote another editor who hailed the departure of one of our greatest expeditions to the Southern Seas with the words, "We may wait, it is true, and consign to centuries to come, the toils, the glories and the hopes of science, or we may rely on an easy effort distributed over length of years for the accomplishment of much that vigorous exertion might now effect". He continues, "There are secrets of Nature we would fain see revealed while we yet live in the flesh—resources hidden in her fertile bosom for the well-being of man upon Earth we would fain see opened up for the use of the generation to which we belong. But if we would be enlightened by the one or benefited by the other, we must lay on power both moral and physical without grudging and without stint". He makes a more encouraging reference to the poet who wrote:

"Nimm die Zögernde zum Rath
Nicht zum Werkzeug deiner That"

These admonitions appeared in the *Quarterly Review* 120 years ago, and they are as timely and well-intentioned as the new cautions.

Extensive investigation of the South Atlantic Ocean by the German *Meteor* Expedition in 1925–27 did much to advance marine science, and there is little doubt that concerted effort with our new techniques in the Indian Ocean will meet with similar success provided we give ourselves as much time to learn from it, and avoid too much organization as a pattern of things to come. Perhaps the new venture follows too soon on the International Geophysical Year surveys, but the organization and the problems are being approached very carefully, by the research-minded scientists as well as those who see the need for some campaigning.

The planning and operating of programmes is being done by national committees for oceanic research. Growing interest in them has already sent research ships from the United States and the U.S.S.R. to the Indian Ocean, and useful observations are also being made by H.M. survey ships and Royal Australian Navy frigates. At the peak of activity, covering the north-easterly and south-westerly monsoons of 1962–63, there may be as many as twelve ships working in fairly close co-operation. It is expected that a ship from the United Kingdom will be there, and that she will play an effective part in the programme as well as serving as a base for scientists whose work is likely to be advanced by special observations in Indian Ocean conditions.

The Special Committee on Oceanic Research chose Copenhagen and Helsinki for its meetings because many marine scientists were already at Copenhagen to take part in intergovernmental discussions arranged by Unesco, and at Helsinki to participate in the scientific meetings of the International Union of Geodesy and Geophysics. There is much careful and detailed work to do. Those taking part will be heartened by the recent announcement from the White House of active support to the U.S. contribution for which the National Academy of Sciences–National Research Council is scientifically responsible.

G. E. R. Deacon

[1] See *Nature*, **180**, 894 (1957).

# WATER RESOURCES IN THE UNITED STATES

THE pressure of growing populations with rising standards of living increasingly brings water into focus as a basic raw material in need of both study and conservation. Nowhere is this realized more fully than in the United States, where development in a wide range of activities is at present limited by water supplies. As a result of Senate Resolution No. 48 of the eighty-sixth Congress of the United States a Select Committee on National Water Resources was set up in 1959 to study the extent and character of water resources activities required to provide the future quantity and quality of water needed for all segments of the economy in the United States.

Headed by Senator Robert S. Kerr from Oklahoma, this Committee has now been at work during the past year and has within a short space of time produced an impressive array of reports on a wide variety of hydrological topics*. Although confined to the United States, many of these reports have relevance to water problems that the United Kingdom and the Commonwealth face, and it is the aim of this review to indicate the coverage of the reports so far available together with their main conclusions. The speed with which the Select Committee has produced a great body of useful basic material is notable, and this has been achieved by the Committee calling upon existing authorities and organizations to provide the appropriate hydrological information.

* Reports available in June 1960 and printed for the use of the Select Committee on National Water Resources of the United States Senate pursuant to Senate Resolution No. 48 of the Eighty-Sixth Congress. Government Printing Office, Washington:

No. 2, Reviews of National Water Resources during the Past Fifty Years, October 1959; No. 3, National Water Resources and Problems January 1960; No. 4, Surface Water Resources of the United States, January 1960; No. 7, Future Water Requirements for Municipal Use, January 1960; No. 9, Pollution Abatement, January 1960; No. 12, Land and Water Potentials and Future Requirements for Water, December 1959; No. 13, Estimated Water Requirements for Agricultural Purposes and their Effects on Water Supplies, December 1959; No. 16, Flood Problems and Management in the Tennessee River Basin, December 1959; No. 19, Water Resources of Alaska, January 1960; No. 20, Water Resources of Hawaii, January 1960; No. 21, Evapo-Transpiration Reduction, February 1960; No. 22, Weather Modification, January 1960; No. 23, Evaporation Reduction and Seepage Control, December 1959; No. 24, Water Quality Management, February 1960; No. 25, River Forecasting and Hydrometeorological Analysis, November 1959; No. 26, Saline Water Conversion, November 1959; No. 27, Application and Effects of Nuclear Energy, December 1959; No. 28, Water Resources Research Needs, February 1960.

As a result the reports fall into a number of natural groups largely related to the reporting department, the stage being set by No. 2, where the Legislative Reference Service of the Library of Congress reviews and summarizes the work of more than 20 commissions or committees which have investigated national water policies and problems in the United States during the past fifty years. With few exceptions previous study groups have paid little attention to the questions which the Senate has placed before the present Select Committee; notably, "How much water development is needed? When is it needed? Where is it needed? What should be the pattern of water development? What amounts of water will be required in relation to various levels of population and economic activity? What are the economic limits of water development?"

One of the first authorities that the Committee turned to for basic information was the Geological Survey; this department has prepared reports Nos. 3 and 4 dealing with the national water resources, both surface and underground, and related problems. In addition to furnishing quantitative information showing the use of water, the availability of water, and the amount of storage required to maintain certain rates of river-flow, report No. 3 identifies and discusses water problems in the fields of supply, variability, distribution, natural quality, pollution and floods. The report indicates that almost every part of the United States faces current or potential water problems. An estimated water use in 1960 of 250 thousand million gallons of fresh water a day is projected to rise to 600 thousand million gallons a day by 1980. This fortunately still falls considerably short of the estimated manageable supply of 1,160 thousand million gallons a day, but it does mean that in many areas much water will have to be used over and over again and that pollution abatement becomes critical. Report No. 4 presents in tabular and map form the surface water resources of each State. Data on average, maximum, and minimum discharges in cubic feet per second, and average annual run-off in acre-feet at key-points within each State are given, together with a very interesting set of maps showing

comparative river-flows. This report really summarizes in a most effective way the annual surface water supply papers of the United States.

Reports Nos. 7, 9 and 24 emanate from the Department of Health, Education and Welfare; these deal with the future water requirements for municipal use, pollution abatement, and water quality management respectively. At the turn of the century about 30 million people in the United States had a municipal water supply and were using some 3 thousand million gallons a day. In 1954, the number supplied had increased to more than 100 million people and these were using 16 thousand million gallons of water daily. This is an average rate of nearly 150 gallons *per capita* a day with maxima of more than 200 gallons a day per person in some towns. These figures of course include both trade and domestic use. Report No. 7 shows that with medium rates of growth of population this level of water consumption will probably rise to 29 thousand million gallons a day by 1980 and 43 thousand million gallons a day by the year 2000. With a high rate of population increase the figures would be 33 thousand millions by 1980 and 58 thousand millions by the year 2000. Assuming that certain stimulants of municipal use continue to occur and taking a maximum population increase, a ceiling of 43 thousand millions by 1980 and 88 thousand million by the year 2000 is theoretically possible. Daily consumption *per capita* might then be of the order of 225 gallons. If cities are to continue to supply their residents with adequate supplies of clean fresh-water much forethought is clearly going to be necessary.

Report No. 9 from the same department deals with pollution, and directs attention clearly to the magnitude of the problem which confronts the United States in its search for clean water. In addition to the normal problems of disposal of sewage and ordinary industrial wastes, many new types of pollution have recently appeared. Synthetic organic chemicals, radioactive substances and newly identified viruses and other disease-producing micro-organisms are the problems of principal concern.

The third report from this Department, No. 24 in the series, deals with water quality management. This discusses water quality and quantity requirements for various purposes, the limitations of streams as waste carriers, and the economics of water pollution. It points out that the growing demands for water and limitations on developable supplies inevitably imply a greater re-use of river-flows, and it further emphasizes that many areas are rapidly running out of clean water.

The next group of reports, Nos. 12, 13 and 28, have been prepared by the Department of Agriculture, and these deal respectively with land and water potentials and future requirements for water, estimated requirements of water for agricultural purposes, and the needs of water resources research. Report No. 12 confirms that the use of water for agricultural purposes is the largest consumer of water in the United States, even though in recent years withdrawals of water for industrial purposes have exceeded those for irrigation. Generally, however, more than half the water withdrawn for irrigation is consumed by evaporation and transpiration, while less than a tenth of the water withdrawn for industrial purposes is lost. Agriculture also becomes of increasing significance in the water problem because of the growing trends in the United States to irrigate the humid areas as a means of increasing yields of crops. The report shows that the diversion and storage of water for irrigation in 1954 was 195 million acre-feet, of which 115 million acre-feet were actually used for irrigation. On the basis of projections of medium-level populations the Department of Agriculture estimates that requirements of water for irrigation in 1980 will amount to 181 million acre-feet for storage and diversion with 114 million acre-feet actually used for irrigation. This somewhat surprising and welcome reduction emerges as a result of assuming the application of known improved methods of water management. Failing, however, the adoption of these improved methods of storing, transporting and using irrigation water, enormous additional amounts of water would be required.

In addition to the direct use of water for agricultural production there are a number of other programmes in the United States for the conservation and development of agricultural land which affect water resources. These include the run-off and water-flow retardation schemes, soil erosion prevention, and watershed improvement. Report No. 13 deals with the implications of these: at present programmes for land treatment have been put into effect on some 18 per cent of the crop-land, pastures and woodlands. This should increase to 32 per cent by 1980 and 52 per cent by the year 2000. These programmes will, it is thought, deplete downstream flows by 4·5 million acre-feet in 1980 and by 7·8 million acre-feet in the year 2000. These amounts are small in comparison with the requirements of irrigated agriculture and are not thought to be problematical. Report No. 28, also prepared by the Department of Agriculture, lists the research which will be needed if the best use of water is to be made in the production of essential agricultural and forest products. This report shows a welcome awareness of the importance of water as a prime raw material worthy of care and conservation.

The next group of reports, Nos. 22 and 25, emanate from the United States Weather Bureau and deal with weather modification, river forecasting and hydrometeorological analysis. In the broadest sense weather modification includes all man-made changes in natural conditions of weather and climate. It applies to a wide variety of atmospheric phenomena ranging in scale from micro- to macro-conditions. Report No. 22, however, deals primarily with cloud modification as a means for increasing rainfall. As only 5–15 per cent of the available moisture in a cloud system reaches the ground during a rainstorm, it is obvious that even a slight increase in the amount of precipitation could have a tremendous effect on water supplies. It has been held that this possibility therefore justifies the very considerable expense that has so far been incurred in artificial rain-making. To date, however, the Weather Bureau has had to admit that much uncertainty still remains in the results of cloud seeding and in the practical utility of present methods of artificially increasing rainfall. The Weather Bureau also has the responsibility of forecasting river-flows for the purpose of reducing flood damage and the prevention of loss of life from floods, and report No. 25 deals with future needs and possibilities for improvements in this service as a means of increasing the usefulness of available water supplies.

A further group of reports, Nos. 19–21, 23 and 26, emanate from the Department of the Interior. Nos. 19 and 20 are straightforward accounts of the water

resources of Alaska and Hawaii respectively. These two States have been considered separately from the continuous area of the United States as so many of their water problems are different. The report on Alaska shows that four main problems will hinder water supply development in the far north: these are the winter freeze and permafrost, the presence of glacial flour, impervious consolidated rocks limiting ground-water storage, and problems of waste disposal in the winter months. It is interesting to note that irrigation is being employed in Alaska and that eventually this should grow to 50,000 acres. The potential of water-power is enormous and more than 13 million kW. could be developed at some 168 sites. In Hawaii the main problem appears to be the unequal distribution in both time and place of the precipitation, and this produces recurring water shortages despite the abundant rainfall. Immediate problems are not, however, incapable of solution.

Reports Nos. 21 and 23 may be taken together as these deal respectively with evapo-transpiration reduction, and evaporation reduction and seepage control. Evapo-transpiration is the transfer of water to the atmosphere from plants and other surfaces as a result of normal evaporation of precipitation, and also transpiration, which is the release of water vapour to the atmosphere in the process of photosynthesis. The Department of the Interior reports that opportunities for improved yields of water through management of vegetation appear favourable on some 15 per cent of the area of 17 western States; with a possibility of increasing water-yields in amounts ranging from about 1 to 6 inches in certain forest areas of heavy precipitation to as low as 0·25 in. in the pinon-juniper regions. No increase at all is possible on the semi-arid lands covered with grass and shrub which make up the greater portion of the south-west United States. The Department has also investigated the effect of losses from phreatophytes, the water-loving 'well plants' which cause a major loss of water along rivers in the western States. These cover some 16 million acres and discharge 20–25 million acre-feet of water into the atmosphere annually. By controlling the phreatophytes it should be possible to save some 6 million acre-feet of water by 1960 and up to 12 million acre-feet by the year 2000. Much water is also lost from irrigation works by seepage and evaporation, and it is estimated that improvements in irrigation water conveyance works could save up to 4·5 million acre-feet by 1980.

Report No. 26 is concerned with the increasingly important topic of saline water conversion. The search for means of tapping this obvious unlimited source of water has taxed the ingenuity of man for many centuries, and practical methods for desalinization of ocean water in small quantities have been used on ships for more than a century. Attention has more recently been turned to the construction of large-scale land-based plants. A programme of research was launched in the United States in 1952 and further enlarged in 1955. In 1958 Congress authorized the construction of large-scale experimental demonstration plants, and this programme is now under way. In spite of rising prices the cost of converting sea and brackish water to fresh is continually being lowered. Before the Second World War it stood at 4 dollars upwards per thousand gallons: the new experimental plants should be converting saline water to fresh for less than a dollar per thousand gallons, while processes now in the laboratory stage give promise of conversion-rates below 50 cents per thousand gallons. This report details all the current and proposed fundamental research, and is a real mine of information for those concerned with this problem.

Finally, there are two other reports of interest: from the Tennessee Valley Authority comes No. 16 on flood problems and management in the Tennessee River Basin, and from the Atomic Energy Commission there is No. 26 dealing with the application and effects of nuclear energy. The flood problem is the converse of the problem of water shortage and, like the United Kingdom, the United States has had in most parts a multiplicity of authorities dealing separately with problems which are really integrated. Programmes for run-off and water-flow retardation and prevention of soil erosion have been the responsibility of the Department of Agriculture, downstream flood-control works have been in charge of the Corps of Engineers: reclamation work falls to the Bureau of Reclamation. Other authorities are responsible for the development of power, public water supply, irrigation and so on. In the Tennessee River Basin, however, there is an example of an integrated river basin development which is a model of its kind. Here the Authority covers an area of about 40,000 sq. miles and has in its charge all aspects of the hydrological cycle. It was therefore invited to present to the Select Committee any suggestions as to how its experience might be applied to the major water problem in the United States. In report No. 16 it has some interesting recommendations, more particularly in the sphere of development control in flood plain areas.

The particular water needs and problems arising from the development of atomic energy are dealt with in report No. 27 prepared by the Atomic Energy Commission. It is not thought that the requirements and applications of nuclear technology will have any major effect or impact on the development of water resources up to 1980, but the need to recognize the importance of control of water-dispersed waste radio-active products is stressed.

It will be clear that the 600 pages and innumerable diagrams, maps and tables in these 18 reports present a unique and up-to-date picture of the water problems facing the United States. The willingness to look ahead, initially to 1980 and then beyond to the year 2000, at low-, medium- and high-level population densities reveals an awareness of the increasing importance of water in the future; and of the necessity for the present generation to plan for the water needs of the next. In contrast, the British Central Advisory Water Committee in its recent reports was not prepared to look in any detail beyond 1965—which is almost upon us already. A comparison between the two sets of reports also reveals a quite different attitude of mind and approach to essentially the same problems—notably in the case of irrigation in the humid areas. It is to be hoped that the United States Senate Committee reports will become known in Great Britain to all those concerned with the great variety of aspects of the hydrological cycle; notably watershed management, storage of water, supply of water, control of the quality of water, pollution, saline conversion, and irrigation. The reports throw much new light on the increasingly important problem of water conservation.

W. G. V. Balchin

# SHORTAGES OF SCIENTIFIC PERSONNEL IN EUROPEAN COUNTRIES IN PROCESS OF DEVELOPMENT

THE shortage of scientific personnel is common to all European countries, even those which have a long tradition of scientific and technical development backed by powerful resources. Industrialized countries face ever-increasing requirements, while countries which are primarily agricultural and commercial now feel the need to build up their industrial potential in order to compete with the rest of Europe and raise their standard of living.

For such countries the shortage of scientific personnel is particularly acute and raises problems which are most urgent. Chief of these, according to Rector Capelle, director-general of the Institut National des Sciences appliquées, in a recent issue of *European Productivity* (No. 35; April 1960), are: training of teachers of science and technical subjects; broadening of secondary education to include technical subjects; training of engineers; development of scientific research; and the development of university-industry relations.

Industry's demand for scientists and technicians and the generous salaries offered are frequently a severe handicap to the recruitment of science teachers. Teachers' salaries must be raised if the necessary teachers of high calibre are to be found for the rising school population. This applies not only to secondary schools and technical colleges but also to the universities themselves, where too often a professor's salary at the end of his career is no more than that offered to his students on entering industry. A further demand for teachers comes from industry itself, where the old system of training skilled workers and technicians on the job must be supplemented by proper training courses providing the appropriate general education at each level.

In order to persuade more and more young people to become technicians or engineers, and to attract children from families obsessed with the idea of classical education, the traditional secondary education should be broadened to include technical subjects laying the foundations of a technical humanism supplementing, and in no way conflicting, with traditional humanism. In all secondary education, the laboratory and the workshop should now be used as instruments of learning in the same way as books, which still have too much of a monopoly.

In countries in process of development, engineers are trained to a high level and in physical conditions in no way inferior to those prevailing in countries with a long-established technological tradition. Technical faculties or universities training engineers are compelled to make a strict selection of candidates, but this is a good guarantee of success. Many students who cannot find a place in the higher educational establishments of their own country go to a foreign technical university. This additional supply of engineers—sometimes as much as 50 per cent—is not always appreciated.

The methodical increase of means of production implies making provision for research. It might seem enough to leave the responsibility for research to the countries best equipped for it. This would in the end be detrimental to young industries. The organization of scientific research will become a vital necessity for all countries and will call for the combination of all human and material resources; the Government, the universities and industry must all co-operate, and here the university can play a leading part without any loss of dignity or independence.

Applied scientific research is one of the links to be established between universities and industry, two worlds which are much too far apart in most countries in process of development. Industry should not be governed purely by a shopkeeper mentality.

# EAST AFRICAN VIRUS RESEARCH INSTITUTE, ENTEBBE

THE report of the East African Virus Research Institute for July, 1958–June, 1959 by the acting director, J. D. Gillett, shows that the approach to virus studies is still essentially ecological—using the word in its widest sense to cover interaction between virus host and environment (Pp. ii+49. (Nairobi: Government Printer, 1960.) 5*s*.). Attention has again been confined to those viruses which are transmitted to man by arthropods; 60 different viruses are now known or suspected of being arthropod borne. Mosquitoes are involved or suspected in the transmission of no less than 48 of these, and man has been implicated in 47 of them. Twenty-six of these viruses have been found in Africa, 14 of them coming from East Africa itself. Eleven of the 14 East African viruses were originally isolated in the Institute, three of them during the current year.

The isolation of viruses from the living vertebrate host, including man, is often missed because of the transient nature of the viræmia. Nevertheless, in addition to several strains isolated during an outbreak of yellow fever in the Congo, five isolations have been made in East Africa from man; three of these in the period under review were from members of the Institute staff. Three of the five East African isolates appear to be new agents, or at least new strains. One of the others was an accidental laboratory infection of Wesselbron virus, and opportunity was taken of keeping full clinical and physiological records of the course of this very unpleasant disease.

More than 20,000 wild mosquitoes were caught and processed in the laboratory, but no virus isolations were made. This is the second year in which this usually fruitful source of virus has been unproductive.

The work started the previous year on a possible vaccine against Nairobi sheep disease, the virus of which apparently involves man as well as sheep, has been further developed. The first field-trial with the new experimental vaccine carried out in collaboration with the East African Trypanosomiasis Research Organization at Sukulu has fully justified earlier hopes.

Antibody surveys in local residents have been extended by increasing the number of sera tested and by widening the spectrum of viruses used. Positive reactions have been found in all but one of the ten agents used. Surveys of birds were started again, but no positive results have been obtained.

Results of the antibody surveys on a large number of hippopotamus sera have given unexpected results, and are difficult to interpret. A high proportion of the sera has given positive neutralization against all five viruses tested.

Laboratory studies on the viruses of Rift Valley fever, encephalomyocarditis, Semliki Forest fever and yellow fever have been carried out in rodents, primates, birds and insects respectively.

The outstanding event of the year was the completion of the 120-ft. steel tower in the Mpanga Forest. This has brought about a considerable expansion in the work on the biology of mosquitoes and other blood-sucking flying insects.

The successful transmission of virus by mosquitoes depends partly on an adequate extrinsic incubation period during which the virus multiplies in the insect and reaches the salivary glands. Thus, if a species of mosquito is short-lived in Nature, it is unlikely to take part in virus transmission, even though it may be an efficient transmitter in the laboratory. It is of great importance to be able to tell the age of wild-caught mosquitoes. While this can be done in certain species with considerable accuracy by dissection, it would be an advantage if such determination could be correlated with external features. Work on this problem, started in 1951, was taken up again two years ago and has been continued and extended.

## THE CARNEGIE UNITED KINGDOM TRUST

THE forty-sixth annual report of the Carnegie United Kingdom Trust, covering the year 1959, the fourth of the present quinquennium, is largely an account of the working out of ideas, schemes and projects approved and announced some time ago*. During the year, however, the Trustees have noted signs of voluntary work being richly leavened out of all proportion to the contributions made by the Trust, but while, as public responsibility for education at all stages has increased, the opportunities for the Trust have correspondingly diminished, the Trustees continue to find voluntary efforts that are unsupported, or inadequately supported from public funds. The quinquennial provision of £50,000 in aid of museums and art gallery development has been absorbed proportionately over the four years to the end of 1959, and it seems likely that the uncommitted balance will suffice to meet claims upon it in the remaining period. Assistance to the Museums Association was continued, but last year's grant was reduced to £1,200, in the expectation that the Association will thereby be encouraged to become self-supporting. A gratifying feature was the number of museums, earlier grant-aided by the Trust, that put in hand substantial schemes for enhancing the display of their collections and for improving their services generally.

Much of the work of the Museums Joint Committee is concerned with improved museum lay-out, and during the year a further twenty reports were commissioned in connexion with applications for grants for methods whereby collections may be more attractively and dynamically displayed; and arising from the recommendations of these and earlier reports grants totalling £9,065 were offered to seventeen museums and art galleries. After considering in consultation with the Ministry of Works, the Ancient Monuments Board, the National Museum of Antiquities and the Society of Antiquaries, a proposition to rebuild a medieval structure at the north-east of the main block of Iona Cathedral to serve as a lapidarium to house the numerous historical sculptures in and around the Cathedral, the Trustees approved a grant of £6,000 to rebuild the medieval infirmary to be used eventually as a museum of cathedral treasures, but meanwhile to house the most important stones until a permanent lapidarium is provided.

Up to the end of 1959 the Trust had received 1,287 applications from the committees of pre-war village halls, serving small rural communities, for assistance in meeting the cost of renovating and equipping their premises, and of these, 1,031, representing a total commitment of £131,588, had by that date been successful. In consultation with the Scottish Council of Social Service the Trustees have also adopted a modified scheme which will make it easier for such small communities as those on remote islands off the coast of Scotland to embark on the improvement of their halls. Grants totalling £9,190 were promised during the year towards the building of five new village halls in Eire, and under the scheme for providing film projectors and screens for the use of crofting communities, equipment was installed or is about to be installed in thirteen places in the Highlands. A review of progress in the seven schemes for extending into rural areas the advice services available in urban districts through the Citizens' Advice Bureaux, indicates that the experiments have been worth while and the services will be continued when the Trust's grant terminates. The committee set up to study and discuss the development problems of new communities also made satisfactory progress, and the report emphasizes the primary need for leadership in such communities. The National Federation of Community Associations was promised a grant of £5,500 to finance a two-year project for training full-time community centre wardens. The first group of trainees completed their course in March 1959, and a second group started a similar course in the autumn of 1959. Annual grants were also made to the various national councils of social service, and a grant of £6,500 in annual instalments towards the establishment of the North Ireland Association for Mental Health, a like amount being provided by the Government of Northern Ireland. A grant of £2,500 over the first two years was offered towards the cost of an experimental publicity campaign for three years by the Liverpool United Voluntary Organization for the maintenance of voluntary social service.

In education, the Trust supported 173 bursaries, totalling £454, with the Field Studies Council

* Carnegie United Kingdom Trust. 46th Annual Report, 1959. Pp. 50. (Dunfermline: Carnegie United Kingdom Trust, 1960.)

and the Scottish Field Studies Association. Most of the grants in 1959 for field work in archæology were towards the fees and expenses of visiting experts acting as directors of excavations and other field work. Up to 1958 astronomy was the only purely physical science which had qualified for inclusion in the Trust's scheme for the development of amateur scientific activities out of doors. During 1959 the Advisory Committee recommended the inclusion of meteorology, and the Trust has made a grant of £1,000 over 1959 and 1960 towards a scheme prepared by the Royal Meteorological Society. The work during the first season of the Conservation Corps showed a substantial untapped reservoir of potential public service, and the Trust is meeting the cost of a scheme for providing in the Queen Elizabeth Forest Park a hill-top view-point with indicator and shelter at the highest point on Duke's road in the Trossachs and a hill-top pavilion near Aberfoyle. A grant of £12,750 was made to the Homecrafts Advisory Association for the Disabled, established by the Central Council for the Care of Cripples, to finance the development of the Association over five years.

## THE ONTARIO RESEARCH FOUNDATION

THE annual report of the Ontario Research Foundation for 1959 includes a list of papers published during the year and a list of the professional and technical staff (Pp. 40. Toronto: Ontario Research Foundation, 1960). Appended are lists of grants for postgraduate studies in science, 1959–60.

In biochemistry, work continued on long-term projects such as the effect of dietary fats on blood cholesterol-levels, the development of a self-contained domestic sewage unit, the evaluation of spruce bark as a source of tanning materials and drilling mud additives, and the reclamation of purified cellulose from waste paper. In chemistry most of the long-term research and industrial fellowships in wood chemistry were concerned with lignin, including work on the chemistry of vanillin and related compounds, development of a new method for the removal of sugars, based on the solubility in organic solvents of a tertiary amine salt of the lignosulphonates. A reasonably clear understanding of the tanning potentialities of lignin has been obtained and no further work as a major project is contemplated. The activities and staff of the Physical Chemistry Division increased considerably, and basic research was continued on stereo-specific catalysts for the polmerization of 1-butene.

In engineering and metallurgy ore dressing work materially increased, and basic research included trials on five iron ores of an improved flash roaster, evaluation of means for improving electrostatic separating machines and a study of the fundamental properties of magnetites and hæmatites. A major project in process metallurgy was a continuation of the jet smelting programme, and basic research in physical metallurgy was largely concerned with a fundamental study of the fatigue of metals. A possible method for measuring residual microstresses was indicated as well as for studying the ductile–brittle transition zone in certain alloys. An extensive examination of steel and cast-iron mains and services formed part of a more general investigation of safety in natural gas distribution systems for the Ontario Government.

In parasitology, research comprised a survey of parasites of trout in certain lakes in Algonquin Park and an investigation of the mode of transmission of two tapeworms in trout; investigation of selected roundworms; investigation of life histories of blood parasites of birds and the possibilities of their transfer; and blood-sucking insects in relation to their feeding habits and the importance of insects in transmitting blood parasites.

In the Physics Department, basic research centred around the production and application of electron beams. Development of the atmospheric electron probe with X-ray spectrometer continued. An optical bench for investigating electron beams of wide cross-section was designed and its construction almost completed, and construction of a fluorescent probe for the University of Toronto Institute of Aerophysics was completed. In physiography an intimate knowledge of the soils and climate of Ontario continues to be built up, and in studies of crops a heat index was derived from growth-chamber results in Indiana and applied to thirty years records at the Central Experimental Farm at Ottawa. In textiles, continued research on wool sought new and improved methods of increasing resistance to alkali and reducing agents and of lowering felting properties. In the modification of cellulose fibres to improve crease resistance and fabric stability good progress has been made with the use of epoxy resins and of vapour phase reactions with epichlorohydrin.

## SOCIAL DEVELOPMENT THROUGH FAMILY AND HOME

THE report of the conference on "Social Development through the Family and Home," which was held at St. Hugh's College, Oxford, during September 7–25, 1959, under the auspices of the Secretary of State for the Colonies, has now been published (Pp. ii+94. (London: H.M. Stationery Office, 1960.) 4*s*. net).

The conference, the third in a series dealing with social development in Britain's oversea territories, was attended by representatives of 28 territories and by participants from four international organizations, from the Colonial Office and from the Secretary of State's Advisory Committee on Social Development.

The report directs special attention to the role of the family as a basic unit of society, and to the need for Governments to adopt a social development policy to support it in conditions of rapid social and economic change. It recommends the establishment, in overseas territories, of 'ministries of social development,' supported by departments or services with

trained workers, and the setting up of social development councils to advise Governments. Among its recommendations on the training of social service workers, the report points out the need, in rapidly changing societies, for trained workers. Their training should for preference take place in the territories in which they will work, although it recognizes the advantage of some additional training overseas for occupants of the highest posts.

More specifically, the report recommends the introduction into schools of education for family life, so that both boys and girls may learn to play a happy and considerate part as young people and later as adults in the circle of their families and friends. Informal education among adults "is one of the most important ways of establishing a right relation between formal schooling and social development".

Turning to food, nutrition and health, the report advocates the study of dietary habits, consumer education, and community development campaigns to improve health and hygiene. Recognizing the importance of housing, it calls for advance consideration of the needs and customs of those who will live in houses which are being planned. Against the background of economic development, facilities for employment of women, the establishment of day nurseries, industrial welfare, social security and recreational facilities are considered to assume increasing importance. The report describes as important the availability of advice to families and as urgent the need for study of the legal status of the family, with particular reference to the new patterns of family life emerging as a result of industrialization and the growth of towns.

## LABORATORY-TEACHING OF LANGUAGES

WITH the aid of mechanical and electronic devices, students may now use entire class periods for practice in reading, speaking and comprehension during language laboratory sessions (Industrial Bulletin of Arthur D. Little, Inc., No. 373; March 1960).

The language laboratory system emphasizes the 'hearing and speaking' approach which is endorsed by the Modern Languages Association of the United States and the American Council of Learned Societies. The students are exposed to their new material on an essentially private basis. In general, comprehension, performance and speed of learning are greatly improved under the new system.

The Federal Government has recognized the need of language laboratories by providing support for the new facilities, as well as sponsoring scientific research on the mechanics of language teaching. Many colleges, high schools and even private industrial firms have installed language laboratories, where students attend at least once a week. Eventually such laboratories may become so widespread that college entrance requirements in language will include oral as well as written tests.

In class, students sit in semi-sound-proof isolation booths provided with earphones; a master tape-recording is played, and the teacher may project slides or other visual aids simultaneously. Master tapes may be pre-recorded and catalogued and filed like library books; special text-books and exercises accompany each tape. Well-placed pauses in the tape allow the student to repeat phrases; the whole class is monitored by the teacher, who may tune in to make corrections of inflexion and pronunciation. Special instruction may be given to individuals needing it most, thus solving in part the familiar class-room problem of dividing teaching time equally among students.

Language laboratories are not intended to replace teachers, but to supplement them. Although, under the new system, students can progress faster than under the old, and will have more opportunity to develop their conversational abilities, the teacher will still be needed to explain linguistic structure, to correct errors, and to prepare good master tapes.

## IONOSPHERIC MODIFICATION OF THE RADIO EMISSION FROM JUPITER

By Prof. ALEX G. SMITH, T. D. CARR, H. BOLLHAGEN, N. CHATTERTON and F. SIX

Department of Physics, University of Florida, and Maipu Radioastronomical Observatory, University of Chile

THE sporadic long-wave-length radio-frequency energy emitted by the planet Jupiter is received in the form of brief pulses, the majority of which range from about 0·2 to 1·5 sec. in duration. Occasionally the radiation is very intermittent, with pulses occurring singly, or at most in twos or threes. However, it is more common for the energy to be received in complex bursts of pulses, with each burst lasting from a few seconds to a minute or more.

An experiment reported in 1958 by Gardner and Shain[1] has thrown considerable doubt on the real origin of these details, suggesting that many of the features may originate in the terrestrial ionosphere. In this experiment, antennæ spaced 25 km. apart were used for simultaneous reception of outbursts from Jupiter, and the received signals were compared by means of high-speed pen recordings. Although only one completely satisfactory pair of records was obtained, it seemed to demonstrate clearly that there were important differences in the signals received at the two sites.

We have recently repeated this experiment, using a much longer base-line. One antenna was located at the University of Florida Radio Observatory, lat. 29° 39′ N., long. 82° 21′ W., while the other antenna was at the Maipu Radioastronomical Observa-

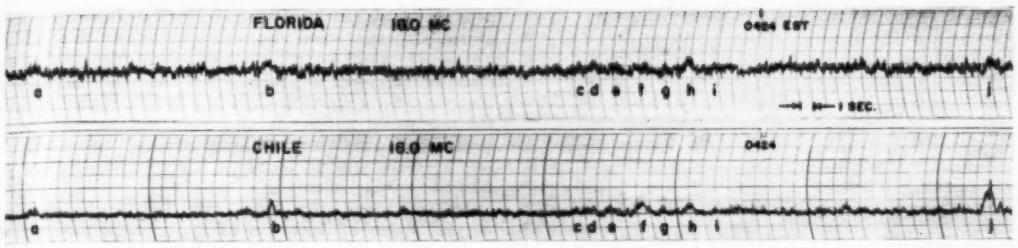


Fig. 1. Period of good correlation from the records of March 29, 1960. Approximate time 4h. 24m. a.m. Eastern Standard Time

tory of the University of Chile, lat. 33° 30′ S., long. 70° 52′ W. The separation of these sites is 7,040 km. Identical receivers were employed at the two Observatories, both receivers being crystal-controlled at 18.000 mc./sec. to eliminate the possibility that differences in the records might be caused by spectral features of the radiation. The recorders were Brush high-speed mechanical oscillographs, having response times of the order of 0·02 sec.; they were operated at a paper speed of 5 mm./sec., which experience has shown is adequate to resolve the details of the pulses.

Simultaneous records of good quality, showing extensive Jupiter activity, were obtained on three nights in early 1960. On March 23 the records overlapped for 31 min., on March 24 for 51 min., and on March 29 for 77 min. Table 1 gives the circumstances of the observations. Time indications were put on all records, either by direct recording of the *WWV* time broadcasts, or by means of a chronometer calibrated against *WWV*; it is believed that the timing was reliable to $\pm$ 1/2 sec. Examination of these records immediately confirms the existence of striking differences in the signals at the two sites. While limited sections of the records show fair correlation of the individual pulses, other sections differ so radically that by actual test it proved to be impossible to correlate the records unambiguously without reference to the time marks. In Fig. 1 is shown a period of moderate activity which is rather typical of a number of intervals during which the pulse-to-pulse correlation was reasonably good. To facilitate comparison, the more obvious correlations have been indicated by means of letters. Fig. 2 shows a short section of the records in which the correlation is much poorer. Particularly interesting is the single pulse *a*, which seems to be replaced in the lower record by a series of five or six peaks.

Careful study shows that many of the grosser differences in the records can be explained as the result of alternate fading or scintillation at the two stations; and by comparing the records during times of sustained activity of Jupiter it is even possible to estimate the period of scintillation for each site. In Fig. 3 is shown a typical example of alternate fading at the two locales. Table 2 shows the mean scintillation period for each station for each of the three nights. It will be seen that on these occasions, at least, there were no significant differences in the periods at the two Observatories.

A number of workers have studied the scintillation of radio stars, which is generally atrributed to drifting irregularities in the ionosphere[3]. The periods reported range from about 8 sec. to 2 min. and are apparently independent of the wave-length at which the observations are made. These workers have also shown that the scintillation amplitudes increase at the lower radio-frequencies, and for sources of small angular extent. Thus, there is every reason to suppose that the observed Jupiter scintillations are of this type. The more rapid scintillations occurring on March 29 may well be associated with the fact that the geomagnetic activity index *K* was larger on that date than on the two preceding dates, since the radio star observations also indicate that the scintillation frequency increases with *K*.

Although portions of the records can be explained as a simple amplitude modulation of the pulse heights, due to ordinary scintillation, other sections seem to

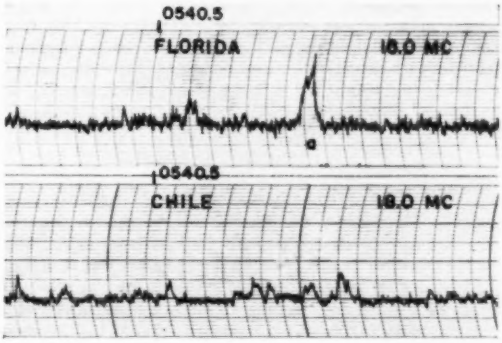


Fig. 2. Period of poor correlation from the records of March 24, 1960. Approximate time 5h. 40m. a.m. Eastern Standard Time

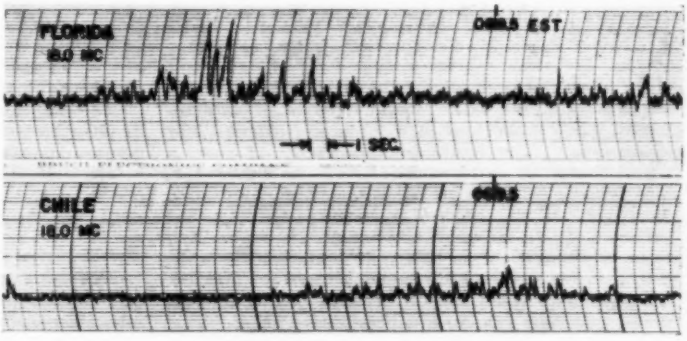


Fig. 3. Out-of-phase scintillations from the records of March 23, 1960. Approximate time 6h. 19m. a.m. Eastern Standard Time

Table 1. Circumstances of the Observations

| Date | Period of observation (E.S.T.) | Range of altitudes of the planet | |
|---|---|---|---|
| | | Florida Station | Chilean Station |
| Mar. 23 | 6h. 06m.–6h. 37m. (a.m.) | 36°–37° | 78°–74° |
| Mar. 24 | 3h. 58m.–5h. 48m. (a.m.) | 25°–36° | 64°–80° |
| Mar. 29 | 3h. 40m.–4h. 59m. (a.m.) | 25°–33° | 64°–77° |

Table 2. Scintillation Periods for the Two Sites

| Date | Mean scintillation period | |
|---|---|---|
| | Florida Station | Chilean Station |
| Mar. 23 | 45 sec. | 41 sec. |
| Mar. 24 | 39 sec. | 43 sec. |
| Mar. 29 | 28 sec. | 29 sec. |

show more complex effects. Even where the scintillations are temporarily in phase at the two stations, the individual pulse shapes are frequently distorted beyond recognition, suggesting the presence of a very rapid scintillation component, with a period comparable to the durations of the pulses themselves, superimposed on the slower modulation described above. This effect seems to be present in Fig. 2, and also in the curious distortion of pulse *b* in Fig. 1.

Measurements of the polarization of the sporadic Jupiter radiation received in the northern hemisphere have been reported by Franklin and Burke[3] and by two of us[4]. Southern hemisphere measurements were made by Gardner and Shain[1]. As the latter remark, it is encouraging that all the measurements have indicated right-hand circular or elliptical polarization, since if the effect were terrestrial in origin the sense of the polarization should be reversed in the two (magnetic) hemispheres. Because the previous experiments were not simultaneous, however, some question has remained as to possible influences of the terrestrial ionosphere.

On March 24, March 29, and April 12 of the current year, we obtained simultaneous polarimeter records at a frequency of 22·2 mc./sec. at the Gainesville and Maipu Observatories, the magnetic latitudes of which are + 41° and − 22°, respectively. Because of an undetected partial malfunction of the Gainesville instrument, a quantitative analysis of the records for the axial ratios of the polarization ellipses is probably unreliable. Qualitatively, however, all the records appear to show right-hand circular or elliptical polarization.

In summary, it appears likely that the basic pulse, of the order of duration of 1 sec., originates from Jupiter, since there are periods of good correlation when nearly identical pulses are received at stations 7,040 km. apart. On the other hand, there is evidence that these pulses frequently are seriously distorted by a rapid scintillation component of terrestrial origin, so that individual pulse shapes must be interpreted with caution in these circumstances. Much of the 'burstiness' of the Jupiter radiation—that is, the tendency of the signals to appear as short trains of pulses—seems to be due to a slower terrestrial scintillation component, which in the present experiments had a period of some 30–40 sec. This latter scintillation is probably identical with that commonly observed for the radio stars. The terrestrial ionosphere does not seem to exert any gross influence on the polarization of the Jupiter radiation, since similar polarizations are observed simultaneously in magnetically opposite hemispheres.

This programme has been supported by the National Science Foundation, the U.S. Army Office of Ordnance Research, and the Office of Naval Research. We are greatly indebted to Dr. Federico Rutllant, director of the National Astronomical Observatory of the University of Chile, without whose assistance the establishment of the Maipu station would have been impossible.

[1] Gardner, F. F., and Shain, C. A., *Austral. J. Phys.*, **11**, 55 (1958).
[2] See, for example, Booker, H. G., *Proc. Inst. Rad. Eng.*, **46**, 298 (1958).
[3] Franklin, K. L., and Burke, B. F., *J. Geophys. Res.*, **63**, 807 (1958).
[4] Smith, A. G., and Carr, T. D., *Astrophys. J.*, **130**, 641 (1959).

# SYNERGISM OF BIVALENT METAL IONS IN TRANSPHOSPHORYLATION

By Dr. JOHN M. LOWENSTEIN

Graduate Department of Biochemistry, Brandeis University, Waltham 54, Massachusetts

Enzyme catalysed transphosphorylations to and from nucleoside polyphosphates constitute many of the key reactions of intermediary metabolism. Most or all reactions of this kind show an absolute requirement for bivalent metal ions, which is commonly met by the ions of magnesium, calcium and manganese. The metal ion specificity of such a reaction may be absolute or relative. In either case, inactive metals may act as competitive inhibitors. Moreover, in the case of relative specificity, the presence of two different, active metal ions may lead to an additive effect, or to an effect which reflects the average of each metal ion taken separately. Finally, the possibility exists that the presence of two different metal ions may lead to a superadditive effect. That the interplay of different bivalent metal ions may be of physiological importance is borne out by a consideration of the concentrations of some metal ions in rat liver. These are magnesium, 9; calcium, 3; and manganese, 0·03 μatoms per gram of fresh tissue[1–3].

Non-enzymic transphosphorylations from adenosine triphosphate to a variety of acceptors also show a requirement for bivalent metal ions[4,5]. The non-enzymic reaction dependent on bivalent metal ions:

$$\text{adenosine triphosphate} + \text{orthophosphate} \xrightarrow{\text{metal}^{2+}} \text{adenosine diphosphate} + \text{pyrophosphate}$$

serves as a model for enzyme catalysed reactions involving nucleoside triphosphates, and provides a chemical basis for the bivalent metal ion requirement of enzyme catalysed transphosphorylations. This reaction proceeds readily in the presence of ions such

Table 1. SYNERGISM OF BIVALENT METALS IN TRANSPHOSPHORYLATION
The reaction mixtures contained 5 μmoles [$^{32}$P]-orthophosphate ($2{\cdot}0 \times 10^5$ counts/min.); 50 μmoles of sodium adenosine triphosphate, *p*H 9; 50 μmoles of diethanolamine–hydrochloric acid buffer, *p*H 9·0; and bivalent metal chlorides as indicated. The final volume was 1·0 ml., temp. 38°, and time of incubation 3 hr. Analyses were performed as described previously (ref. 4)

| | Bivalent metal (μmoles/ml.) $Ca^{2+}$ | $Mn^{2+}$ | $Mg^{2+}$ | Pyrophosphate formed (mμmoles) | Pyrophosphate formed per μmole of first metal (mμmoles) |
|---|---|---|---|---|---|
| *a* | | 20 | | 0 | 0·0 |
| | | | 25 | 0 | 0·0 |
| | | 20 + | 25 | 71 | 3·6 |
| | | 45 | | 103 | 2·3 |
| | | | 45 | 4 | 0·1 |
| *b* | 15 | | | 40 | 2·7 |
| | | | 25 | 0 | 0·0 |
| | 15 + | | 25 | 428 | 28·5 |
| | 40 | | | * | > 30 |
| | | | 40 | 1 | 0·0 |
| *c* | 15 | | | 40 | 2·7 |
| | | 25 | | 7 | 0·3 |
| | 15 + | 25 | | 550 | 36·7 |
| | 40 | | | * | > 30 |
| | | 40 | | 99 | 2·5 |

* Insoluble calcium salts precipitated. Pyrophosphate found in mixture was 1,200 mμmoles.

as calcium, cadmium and manganese, and much more slowly in the presence of ions such as magnesium, beryllium and nickel[4]. Here, too, there exists the possibility that one type of bivalent metal ion may act as a competitive reactant or inhibitor with respect to another type of bivalent metal ion. These possibilities have been studied experimentally using the non-enzymic reaction quoted above. The use together of certain pairs of bivalent metal ions, of which one possesses little or no activity when tested alone and the other possesses high activity when tested alone, leads neither to competitive inhibition nor to an effect which reflects the average of each metal ion taken separately. Instead there can be observed a striking superadditive effect. Results obtained with various combinations of calcium, manganese and magnesium ions are shown in Table 1. In experiment *a* the effects of manganese and magnesium ions were tested separately and together. The first two lines of experiment *a* show that 20 m*M* manganese and 25 m*M* magnesium are insufficient to satisfy the bivalent metal ion requirement of the reaction in the presence of 50 m*M* adenosine triphosphate, and no pyrophosphate is formed. (The symbol m*M* (millimolar), is used to express the concentrations of ions as well as molecules. The terms 'milli-equivalents' and 'milli-ions' are avoided since they may lead to confusion when used for bivalent metal ions in the present context.) However, when these concentrations of manganese and magnesium ions are added together (line 3) they lead to the formation of 71 mμmoles of pyrophosphate. Lines 4 and 5 are controls which show that 103 mμmoles of pyrophosphate are formed in the presence of 45 m*M* manganese, and 4 mμmoles of pyrophosphate are formed in the presence of 45 m*M* magnesium. A concentration of 45 m*M* was chosen for the controls because this was the sum of the concentrations of manganese and magnesium when these were used in combination. It is clear from these results that some, but not all, of the active manganese ions can be replaced by the much less active magnesium ions without loss of activity per μmole of the remaining manganese ions (cf. last column of Table 1). The two metals can therefore be said to work in synergism. The results obtained with calcium and magnesium ions (experiment *b*), and with calcium and manganese ions (experiment *c*) are similar. The synergism is most pronounced when the ion which possesses relatively low activity is present in a concentration which is equal to one-half or more of the concentration of adenosine triphosphate.

Under conditions of *p*H and adenosine triphosphate/bivalent metal ratio which are suitable for studying the catalytic activity of calcium, manganese and magnesium ions, others, such as barium and strontium ions, give precipitates. The observations reported in Table 1 show that very small concentrations of highly active ions, such as calcium, can be demonstrated to possess catalytic activity in the transphosphorylation reaction if relatively large amounts of, say, magnesium ions are also present. It is therefore possible to test very low concentrations of barium and strontium ions for activity by taking advantage of the sparing action of magnesium ions. The results of such an experiment (Table 2) show clearly that barium and strontium ions can also be classified as highly active in the catalysis of the transfer reaction. This finding could not have been anticipated from previous results[4], which tended to indicate that metal ions active in the non-enzymic reaction have an ionic radius between 0·8 and 1·0 Å.

A bivalent metal ion which possesses little or no catalytic activity on its own can also be classified according to its ability to participate in a synergistic partnership with a highly active metal ion such as calcium. An experiment designed to demonstrate this is shown in Table 3. The ions are listed in descending order of effectiveness as determined by the difference between the activities observed in the presence and absence of calcium ions. The results indicate that some metal ions are very efficient in synergistic partnership with calcium ions, even though they have virtually no activity on their own. Other metal ions are much less effective, whether tested in the presence or absence of calcium ions. An examination of the results obtained with the first four of the metal ions listed in Table 3 also shows that the effectiveness of a metal ion in the synergistic reaction is not directly related to its effectiveness when it is used alone. Thus the increase in the rate of transphosphorylation when calcium ions are added to manganese ions is of the same order as that which is observed when calcium ions are added to magnesium ions. Yet when tested alone, the effectiveness of manganese is much greater than that of magnesium ions. The same considerations apply to a comparison of cadmium and cobalt ions.

The results of Tables 2 and 3 may be summarized as follows: the alkaline earth ions calcium, strontium

Table 2. ACTIVITY OF SOME BIVALENT METAL IONS IN THE PRESENCE OF 40 m*M* MAGNESIUM IONS
The complete reaction mixture contained 5 μmoles diethanolamine [$^{32}$P]-orthophosphate, *p*H 9·0 ($2{\cdot}76 \times 10^5$ counts/min.); 50 μmoles of diethanolamine adenosine triphosphate, *p*H 9·0; 40 μmoles of magnesium chloride; and 5 μmoles of a second bivalent metal salt as indicated. The total volume was 1·0 ml., temp. 38°, and time of incubation 3 hr.

| Bivalent metal salt added | Pyrophosphate formed (mμmoles) with $MgCl_2$ | without $MgCl_2$ |
|---|---|---|
| None | 0 | — |
| $MgCl_2$ | 0 | 0 |
| $Be(NO_3)_2$ | 0 | 0 |
| $SnCl_2$ | 0 | 3 |
| $MnCl_2$ | 5 | 0 |
| $CdCl_2$ | 6 | 0 |
| $CaCl_2$ | 42 | 0 |
| $SrCl_2$ | 55 | 0 |
| $BaCl_2$ | 44 | 5 |

Table 3. Activity of some Bivalent Metal Ions in the Presence of 7·5 m*M* Calcium Ions

The complete reaction mixture contained 5 μmoles of diethanolamine [$^{32}$P]-orthophosphate, *p*H 9·0 (7·03 × $10^5$ counts/min.); 50 μmoles of diethanolamine adenosine triphosphate, *p*H 9·0; 7·5 μmoles of calcium chloride; and 40 μmoles of a second bivalent metal salt as indicated. The total volume was 1·0 ml., temp. 38°, and time of incubation 3 hr.

| Bivalent metal salt added | Pyrophosphate formed (mμmoles) with $CaCl_2$ | without $CaCl_2$ | Δ |
|---|---|---|---|
| $MnCl_2$ | 184 | 51 | 133 |
| $MgCl_2$ | 110 | 1 | 109 |
| $CdCl_2$ | 120 | 54 | 66 |
| $CoCl_2$ | 52 | 0 | 52 |
| $ZnSO_4$ | 23 | 5 | 18 |
| $CuSO_4$ | 11 | 0 | 11 |
| $FeSO_4$ | 14 | 8 | 6 |
| None | 5 | — | 5 |
| $HgCl_2$ | 4 | 4 | 0 |
| $NiCl_2$ | 0 | 1 | 0 |

and barium are highly active in catalysing the transphosphorylation reaction. The ions manganese and cadmium are of moderate activity alone, but are of high synergistic activity. The ions magnesium and cobalt are of very low activity alone, but are of high synergistic activity. Lastly, the ions zinc, copper, iron, mercury and nickel possess very low activity alone, and are of low synergistic activity. Beryllium, which possesses very low activity alone (Table 2 and ref. 4) is probably also of low synergistic activity.

The results presented here demonstrate that the catalytic effect of certain pairs of metal ions is neither the average of the effects observed with each metal independently, nor is it additive. Instead, the effect is superadditive. An attempt will now be made to account for this synergism in terms of the existence of various bivalent metal chelates of adenosine triphosphate.

*The postulate that in the active chelate, adenosine triphosphate : bivalent metal ion are as 1 : 1.* It is postulated that the chelates adenosine triphosphate–$Mg^{2+}$ and adenosine triphosphate–$Ca^{2+}$ (stability constants $K_1$) are active in the transphosphorylation reaction, the former possessing a low and the latter a high activity. It is further postulated that the chelates adenosine triphosphate–magnesium–adenosine triphosphate$^{6-}$ and adenosine triphosphate–calcium–adenosine triphosphate$^{6-}$ (stability constants $K_2$) are inactive in the reaction. These postulates are identical with those which were made to account for variations in the rate of the reaction which are observed on varying the adenosine triphosphate/bivalent metal ratio[4]. If the reaction mixture contains adenosine triphosphate and calcium ions such that the ratio of adenosine triphosphate/$Ca^{2+} > 2$, and if $K_2$ for $Ca^{2+}$ is sufficiently great, then the main species in the solution will be the inactive chelate adenosine triphosphate–Ca–adenosine triphosphate$^{6-}$, and the minor species will be the active chelate adenosine triphosphate–$Ca^{2+}$. If, on the other hand, the solution contains adenosine triphosphate and magnesium ions such that the ratio of adenosine triphosphate/$Mg^{2+} \approx 1$, then provided $K_2$ for $Mg^{2+}$ is not much bigger than $K_1$, the solution will contain the low-activity chelate adenosine triphosphate–$Mg^{2-}$ and possibly some of the inactive chelate adenosine triphosphate–Mg–adenosine triphosphate$^{6-}$. Now if magnesium ions are added to a solution containing adenosine triphosphate/$Ca^{2+} > 2$, a shift will occur from the inactive adenosine triphosphate–Ca–adenosine triphosphate$^{6-}$ to the highly active adenosine triphosphate–$Ca^{2-}$ and the low activity adenosine triphosphate–$Mg^{2-}$ (Fig. 1, line i).

**First postulate**

$$\underset{\text{inactive}}{\text{ATP–Ca–ATP}} \xrightarrow{+Mg^{2+}} \underset{\text{high}}{\text{ATP–Ca}} + \underset{\text{low}}{\text{ATP–Mg}} \quad \text{(i)}$$

**Second postulate**

$$\underset{\text{inactive}}{\text{ATP–Ca–ATP}} \xrightarrow{+Mg^{2+}} \underset{\text{low}}{\text{ATP–Ca}} + \underset{\text{low}}{\text{ATP–Mg}} \quad \text{(ii)}$$

$$\underset{\text{low}}{\text{ATP–Ca}} \xrightarrow{+Mg^{2+}} \underset{\text{high}}{\text{Mg–ATP–Ca}} + \underset{\text{low}}{\text{Mg–ATP–Mg}} \quad \text{(iii)}$$

$$\underset{\text{low}}{\text{ATP–Ca}} \xrightarrow{+Ca^{2+}} \underset{\text{high}}{\text{Ca–ATP–Ca}} \quad \text{(iv)}$$

Fig. 1. Various chelates which may be involved in the synergistic effect of two bivalent metal ions on the non-enzymic phosphate transfer from adenosine triphosphate. The word below each chelate refers to the relative rate of phosphate transfer. (Postulate 1 = line i; postulate 2 = lines ii, iii and iv)

*The postulate that in the active chelate, adenosine triphosphate : bivalent metal ion are as 1 : 2.* It is postulated that the chelates Ca–adenosine triphosphate–Ca and Mg–adenosine triphosphate–Ca are highly active in the transphosphorylation reaction, while the chelates Mg–adenosine triphosphate–Mg, adenosine triphosphate–$Mg^{2-}$ and adenosine triphosphate–$Ca^{2-}$ possess low activity, and those consisting of two molecules of adenosine triphosphate and one bivalent metal ion possess no activity. Suppose that to a solution containing adenosine triphosphate/$Ca^{2+} \geqslant 2$ there are added magnesium ions, then there will be a shift from the inactive adenosine triphosphate–Ca–adenosine triphospnate$^{6-}$ to the low activity adenosine triphosphate–$Ca^{2-}$ and adenosine triphosphate–$Mg^{2-}$ (Fig. 1, line ii). The addition of further magnesium ions leads to the formation of the high-activity chelate Mg–adenosine triphosphate–Ca and the low-activity chelate Mg–adenosine triphosphate–Mg (Fig. 1, line iii). Addition of calcium ions instead of magnesium ions leads to adenosine triphosphate–$Ca^{2-}$ and thence to the highly active chelate Ca–adenosine triphosphate–Ca (Fig. 1, line iv). The two postulates are not mutually exclusive, and it is possible that both adenosine triphosphate–$Ca^{2-}$ and Ca–adenosine triphosphate–Ca are highly active in the reaction under discussion. Mg–adenosine triphosphate–Ca would then also be highly active.

The results reported here suggest the following hypothesis to account for the differences in the activity of myofibrillar actomyosin adenosine triphosphatase[6,7] in the presence of calcium, magnesium and potassium ions : myosin contains bound bivalent metals[8] (J. Gergely and B. Vallee have found that myosin contains about 1 atom magnesium and 3 atoms calcium per molecular weight of $5 \times 10^5$ (personal communication)). It follows that myofibrillar actomyosin adenosine triphosphatase also contains bound bivalent metals. In the absence of added bivalent metal but in the presence of potassium ions there is a slow hydrolysis of adenosine triphosphate which involves the formation of an enzyme–calcium–adenosine triphosphate–potassium complex. At low potassium ion concentrations the rate of hydrolysis of adenosine triphosphate is increased by the addition of magnesium or calcium owing to the formation of enzyme–calcium–adenosine triphosphate–magnesium or enzyme–calcium–adenosine triphosphate–calcium. In the presence of added magnesium the system shows great sensitivity to ethylenediaminetetraacetic acid, because ethylenediaminetetraacetic acid

combines preferentially with the bound calcium which is essential for enzyme activity. The formation of enzyme–calcium–adenosine triphosphate–magnesium is prevented by the formation of enzyme–calcium-ethylenediaminetetraacetic acid, and possibly by the subsequent dissociation from the enzyme of some calcium–ethylenediaminetetraacetic acid. In the presence of added calcium the sensitivity of the system to ethylenediaminetetraacetic acid is much lower, and depends in the first instance on the removal of calcium from the solution (and not from the enzyme) by the formation of calcium–ethylenediaminetetraacetic acid. The behaviour of myosin adenosine triphosphatase, although different from myofibrillar adenosine triphosphatase[6], may be accounted for in similar fashion. Myosin adenosine triphosphatase is activated to a much lesser degree by magnesium than is actomyosin adenosine triphosphatase. This reflects a difference in the reactivities of the enzyme–calcium–adenosine triphosphate–magnesium complexes postulated for both hydrolase activities. The postulate is in harmony with the concept that the true substrate of actomyosin adenosine triphosphatase is adenosine triphosphate–calcium or adenosine triphosphate–magnesium, and that free adenosine triphosphate inhibits by being bound to the enzyme without being split rapidly. It does not account for the activation of myosin adenosine triphosphatase which is observed in the presence of ethylenediaminetetraacetic acid under certain conditions[8].

The composite effects of different bivalent metal ions deserve further consideration from the points of view of chelate chemistry and enzyme mechanisms. The importance of such effects in the metabolism of living cells remains to be assessed.

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# FLUORINE COMPOUNDS IN NATURE; THE DISTRIBUTION OF CARBON-FLUORINE COMPOUNDS IN SOME SPECIES OF *DICHAPETALUM*

By Sir RUDOLPH PETERS, F.R.S., and R. J. HALL

Agricultural Research Council Institute of Animal Physiology, Babraham, and Department of Biochemistry, University of Cambridge

THE presence of an organic compound of fluorine in Nature has been known since Marais[1] isolated fluoroacetic acid from the leaves of *Dichapetalum cymosum*, the South African plant called gifblaar, which is poisonous to cattle; fluoroacetic acid was first synthesized by Swarts[2]. Another plant of the same genus, namely, *Dichapetalum toxicarium*, also called ratsbane, has been known for a long time. It grows in Sierra Leone (West Africa) and is responsible for the condition of 'brokeback'[3], a paralytic state in man. The signs described in animals after eating the seeds of this plant resemble so much those produced by fluoroacetic acid that we examined its seeds for a fluorine compound. Fluoroacetate was present only in traces; but we have now isolated two new fluorine compounds from the seeds of *D. toxicarium*. (1) A fluoro-fatty acid constituting most of the toxic material, which has been finally identified as ω-fluoro-oleic acid[4]. (2) The other, present in much less amount, is a saturated long-chain fatty acid possibly having 17 carbon atoms.

Several problems of botanical and other interest arise from these observations.

The question is whether the difference in fluoro compounds in the seeds of *D. toxicarium* as compared with the leaves of *D. cymosum* is a property of the species or whether it is merely due to the storage of the $C_2$ acid by conversion in the seeds to long-chain fluoro-fatty acids. This has led us to examine the leaves and seeds of *D. cymosum* for fluoro-oleic acid and other fluorine compounds.

*D. cymosum seeds.* We are indebted to Dr. R. A. Dyer, chief of the Division of Botany, Department of Agriculture, Pretoria, for sending us some of these seeds, which are stated to be rare, as the plant is difficult to find in fruit.

No trace of fluorine was found in the powdered dried seed after ashing. This proved the absence of any fluoro compound within the sensitivity of the tests. There was not enough material for animal tests to confirm whether these seeds were toxic. It has been stated in the literature that the seeds are toxic so the matter requires further investigation; but it seems evident that any fluoroacetate made is not being metabolized and stored in these seeds as a long-chain acid, as happens in *D. toxicarium*.

*D. cymosum leaves.* These were examined to see if there was present any long-chain fluoro-fatty acid, and if so, whether it was identical with the material from *D. toxicarium*.

The leaves were finely ground in a Cassell mill, and 100 gm. extracted with light petroleum (boiling range 40–60°). The residue after removing the solvent was saponified with methanolic potassium hydroxide, the saturated long-chain fatty acids were separated from acetone solution at − 20° C. and discarded. The acetone solution now contained 0·624 gm. of an oil, mainly of unsaturated acids, and analysis showed that it contained 1·415 mgm. fluorine. When injected into three rats at doses, which in terms of fluorine content would have been toxic as a long-chain fatty acid, no effect was found.

The crude acids were treated according to the recent method given[4] for purifying fluoro-oleic acid. About 20 mgm. of a purified oil was obtained which, when chromatographed by a modification of the

Fig. 1. Ascending chromatogram of unsaturated fatty acids of *Dichapetalum* species, etc., on Whatman No. 531 filter paper impregnated with liquid paraffin, developed with 85 per cent v/v acetic acid/water and demonstrated with iodine vapour

method of Schlenk *et al.*[5], gave the chromatogram shown in Fig. 1. There was present, therefore, a long-chain acid with approximately the same $R_F$ value as fluoro-oleic acid. A long-chain fluoro-fatty acid with an even number of C atoms has a powerful inhibitory effect on citrate metabolism when tested on kidney particle preparations from the guinea pig *in vitro*; 5–10 μgm. of the acid shown to travel as fluoro-oleic acid on the chromatogram was found to inhibit the metabolism of citrate in such preparations. The unknown acid therefore had some of the properties of fluoro-oleic acid, but it differed in that it was not toxic *in vivo* at double the usual fatal dose to rats. The problem requires further exploration; our tentative interpretation is that the leaves of *D. cymosum* can contain in small amounts a long-chain fluoro-fatty acid of 17 or 18 C atoms, which is different from those at present known, though an alternative explanation could be that the crude acids contain a component interfering with toxicity *in vivo*. One batch of *D. cymosum* leaves which we received contained none of this compound. In accordance with previous work by others[6], we have found that the total fluorine content of specimens of leaves varied greatly; from 63 to 270 μgm. fluorine per gm. of dried leaves.

*D. toxicarium leaves.* When these were ground and extracted in the manner as were the leaves of *D. cymosum*, no fluorine was detected on ashing and no trace of a long-chain fluoro-fatty acid.

From the preceding observations it can be concluded that the presence of fluoro-oleic acids in the seeds of *D. toxicarium* is a property of the seeds of this species and not of the seeds of the genus in general. Furthermore, the leaves of this species cannot contain much fluoroacetic acid (Table 1) as no fluorine was found on ashing. There is a distinct indication that there are still more fluorine compounds to be discovered in *D. cymosum* leaves, though it seems unlikely that the toxicity is due to such compounds, which would be present only in traces compared with fluoroacetic acid.

The question also arose whether leaves from a plant of *D. cymosum* grown in England would also contain large amounts of fluorine. Some seeds were kindly brought back from Johannesburg five years ago by Sir Edward Salisbury, who arranged for these to be grown by Mr. Stenning, at the Royal Botanic Gardens, Kew. Two leaves were taken from a plant about 12 in. in height, and were analysed for fluoride after combustion, using a micro-diffusion technique[7]. Relatively large amounts of fluorine were present, 112 μgm./gm. This is in the range of our other figures for total fluorine, and that of the 150 μgm./gm. recorded by Marais[1]. These amounts are to be compared with those of grasses generally, 9–26 μgm./gm. and alfalfa 15–36 μgm./gm.[8]. These authors noted that the fluorine increased as the plants grew and the season advanced; lawn grass was found to contain 16 μgm./gm. dried grass in May and 53 μgm. fluorine/gm. in November. Specimens of tea may give higher values; for example, we found 150 μgm./gm. in a sample of Indian tea.

*Other Dichapetalum species.* During the course of our work, we have been able to examine leaves and stems and roots from several other African species. The scientific interest was reinforced by the possibility that the ingestion of some might account for unexplained deaths of cattle in East Africa. The plants examined have been the following:

Belgian Congo: (1) *D. flabellatiflorum*, Hauman., origin, Germain 0594; (2) *D. thonneri*, de Wild, origin, Louis 6712; (3) *D. thonneri*, de Wild, origin, Louis 13016. East Africa: (4) *D. ruhlandii*, East Africa; (5) *D. mossambicense*, Engl.; (6) *D. stuhlmanii*, Engl.

Tests were made of toxicity to rats mainly by dosing orally. The material was finely ground and administered with rat cake, a method which demonstrates well the toxicity of *D. toxicarium* seeds. None of the plants tested with doses up to about 2·0 gm. proved toxic to the extent of killing the animal. In two cases with *D. stuhlmanii* a temporary depression of the heart-rate was observed. On killing the animals some hours later the citrate contents of the hearts were 326 and 141 μgm./gm. wet tissue and in the kidneys 553 μgm./gm. and 73 μgm./gm. wet tissue respectively, which is to be compared with a normal value of 40 μgm./gm. ± 10.

Table 1. Fluorine Compounds in Leaves and Seeds of *D. cymosum* and *D. toxicarium*

| | Fluorine μgm./gm. dry wt. | | | | |
|---|---|---|---|---|---|
| | Total | $F_t$† | $F_{alk}$·‡ | F. acetate | F. oleic* |
| Leaves | | | | | |
| *D. cymosum* | | | | | |
| Sample (1) | 270 | 36 | 217 | ++ | 0 |
| Sample (2) | 70 | 14 | 9 | + | + |
| *D. toxicarium* | 0 | 12 | 8 | 0 | 0 |
| Seeds | | | | | |
| *D. cymosum* | 0 | | | 0 | 0 |
| *D. toxicarium* | 2,035 | | | trace | ++ |

0 = not measurable.

* Or long-chain fluoro-fatty acid.

† Extracted at *p*H 1·0.

‡ After hydrolysis with conc. sodium hydroxide; represent fluoroacetate and any other alkali labile CF compounds.

As a further control the residues from light petroleum extracts, equivalent to 20 gm. of the leaves of (4) and (5), were injected in acetone into rats; they were non-toxic and without effect upon the citrate content of heart, liver or kidney. This proved the virtual absence of toxic long-chain fluoro-fatty acids.

Since some of these plants have been described as toxic in the literature and presuming their toxicity is due to fluorine compounds, we conclude that the toxicity varies as it does in *D. cymosum* and possibly that the specimens collected for us were harvested at the less-toxic period.

Some of our results are summarized in Table 1.

In addition to those mentioned, we are indebted to Prof. Z. M. Bacq, Dr. M. F. Jurion, director-general, Institut national pour l'Etude agronomique du Congo Belge, and others for help in collecting the plants from the Belgian Congo, to Dr. Galley and staff of the Tropical Products Institute, London, for helping in collecting the West African specimens; to Dr. Greenway for East African plants, and to South African colleagues for specimens of *D. cymosum* leaves. We are also grateful to Miss R. Shawdon and to D. Harbidge for skilful technical assistance with part of the work, and to Prof. F. G. Young for facilities provided.

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# SUSTAINED ACTION OF INJECTED CHELATING AGENTS

By Dr. A. LINDENBAUM and Dr. J. SCHUBERT

Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois

It is noteworthy that, following a single injection of an appropriate chelating agent in subjects whose tissues contain foreign radioactive or non-radioactive metals such as plutonium, thorium, or lead, the subsequent urinary excretion of the metal usually remains well above the control levels for days and even weeks. Since most of the chelating agent administered is rapidly eliminated from the body, this is an unexpected phenomenon which merits investigation. For example, within 6 hr. after injection into rats, about 94–98 per cent of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid is excreted, and at the end of 24 hr. most of the retained dose is in the gastrointestinal tract, with smaller amounts generally diffused throughout the tissue[1]. A very small fraction of the injected dose of the pentaacetic acid persists in the blood for several days[1].

Evidence has been adduced[2] to indicate that these chelating agents combine with the metal to form diffusible chelates within the tissues (though not necessarily intracellular). It is reasonable to assume that the sustained excretion of plutonium after treatment with diethylenetriaminepentaacetic acid[3] reflects the slow but steady diffusion of the metal chelate from the tissues into the blood-stream and thence into the urine. We have made a direct test of this assumption in experimental animals by measurement of the ultrafilterability of the tissue deposits of different radioisotopes following treatment with chelating agents. Ultrafiltration techniques have been useful in determining the state of radioelements in solution[4]. They offer a simple, rapid and effective means for screening potential chelating agents for the treatment of radioelement poisoning, as shown here and elsewhere[5].

Low-pressure ultrafiltration techniques were used[4]. In earlier work described in Fig. 2, suitable pressure was applied by a water pump using 150 mm. mercury pressure. In all other experiments ultrafiltrates were obtained by centrifugation at about 900*g* in a modification of the method of McFarland and Hein[6]. It was possible with the latter technique to obtain 0·1–0·5 ml. of filtrate after centrifugation of a 2–5 gm. sample for 15–45 min. In order to minimize the uncertainties resulting from uneven deposition of colloidal particles due to hydrolysis of plutonium and thorium, we have studied the ultrafilterability of the radioisotopes from blood and tissues taken from intravenously injected animals rather than by *in vitro* addition of the radioisotope to tissue samples. In some cases the chelating agents were added *in vitro* to the tissues of animals previously injected with the radioisotope but not treated with the chelating agents. In other cases, ultrafilterability of the radioisotopes was measured from tissues of treated animals.

In preparation for ultrafiltration, rat bone (the entire weighed calvarium) was fragmented with scissors and crushed in a glass homogenizer with the aid of 5 ml. added saline. The entire weighed liver was either similarly homogenized or was simply cut into small pieces before being placed without saline in Visking bags. The radioactivity of the ultrafiltrate was determined in duplicate or triplicate. The values obtained after treatment with, or addition of, chelating agent were corrected for dilution differences and compared with those from ultrafiltrates of appropriate control tissues.

The effects of diethylenetriaminepentaacetic acid on excretion of plutonium are of interest. The calcium salt (335 mgm./kgm. body-weight as the acid form) or saline was given intraperitoneally to rats three days after a single intravenous injection of a solution containing 35 μgm. plutonium-239 in 1 per cent sodium citrate at *p*H 5–6. The rats and their saline controls were killed at intervals of 1, 2, 3 and 7 days after injection. The ultrafilterability of plutonium from the bone, liver, and plasma was measured directly (see Fig. 1). It is evident that plutonium deposited in the tissues has been rendered diffusible by the pentaacetic acid and that the diffusibility remains ten or more times greater than that of the control animals for at least 7 days after a single injection of plutonium.

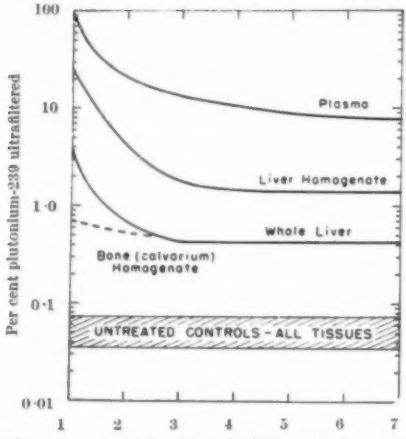


Fig. 1. Ultrafilterability of plutonium-239 from tissues of rats injected intraperitoneally with calcium diethylenetriaminepentaacetic acid (335 mgm./kgm. body-weight as the acid form) three days following intravenous injection of 35 $\mu$gm. plutonium-239

The effectiveness of diethylenetriaminepentaacetic acid relative to ethylenediaminetetraacetic acid in promoting the ultrafilterability of plutonium was tested using plasma and heparinized whole blood. The plutonium was injected intravenously into rats, and 2 ml. of blood was withdrawn 15 min. later. Solutions of the chelating agents were added to the whole blood or to the plasma to provide final concentrations ranging from $10^{-6}$ to $10^{-5}$ $M$. The superiority of diethylenetriaminepentaacetic acid over ethylenediaminetetraacetic acid is shown by the sharper rise in the plutonium content of the ultrafiltrates at concentrations of chelating agent in the range $10^{-6}$ $M$–$10^{-5}$ $M$ (Fig. 2).

It has been previously pointed out[7] that as a result of the mass-action equilibrium, the presence of calcium ions in blood prevents any appreciable chelation of strontium ions. Consequently, ethylenediaminetetraacetic acid and related chelating agents, in the usual therapeutic doses, are useless for treatment of radiostrontium poisoning. In ultrafiltration experiments, the lack of interaction of strontium with ethylenediaminetetraacetic acid was shown

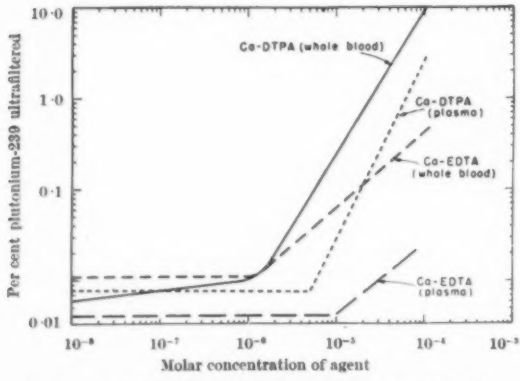


Fig. 2. Effect of concentration of chelating agent on ultrafilterability of plutonium-239 in blood. Plutonium was injected intravenously; chelating agent was added *in vitro*

directly by adding strontium-85 and known amounts of the calcium salt of the latter to rat plasma. At concentrations of ethylenediaminetetraacetic acid as high as $10^{-3}$ $M$ the ultrafilterability of the strontium-85 was unchanged from that of the controls (75 per cent compared with 74 per cent), as could be predicted from the mass-action equations[7]. At a concentration of $2 \times 10^{-2}$ $M$ ethylenediaminetetraacetic acid—a blood concentration unattainable in living animals—there was an increase in the amount of ultrafilterable strontium.

Experiments similar to those described above for plutonium were made with thorium using thorium-234 as tracer. The ultrafilterability of thorium from plasma and tissues obtained from rats injected intravenously with thorium-234 was measured in the presence of $10^{-4}$ $M$ chelating agent added *in vitro*. The marked superiority of diethylenetriaminepentaacetic acid relative to ethylenediaminetetraacetic acid and other chelating agents is shown in Table 1. A similar superiority of the former over the latter in accelerating the excretion of thorium from animals has also been demonstrated[8]. Such results confirm what could be anticipated by comparison of the constants[9] for formation of the 1 : 1 chelates (log $K$(Th-EDTA) = 23·2; log $K$(Th-DTPA) = > 27).

Table 1. Effect of Chelating Agents added *in vitro* on the Ultrafilterability of Thorium-234 from Rat Tissues

| Chelating agent (calcium salt) | Thorium-234 content ultrafiltered (per cent) | | |
|---|---|---|---|
| | Plasma | Liver | Bone |
| Saline controls | 0·26 | 1·2 | 0 |
| Ethylenediaminetetraacetic acid | 2·3 | — | — |
| 1 : 2-*Bis*-[2-di (carboxymethyl) aminoethoxy] ethane | 3·9 | — | — |
| 2 : 2′-*Bis* [di (carboxymethyl) amino] diethyl ether | 4·2 | — | — |
| Diethylenetriaminepentaacetic acid | 26 | 35 | ~10 |

The plasma and tissues were obtained from rats killed 15 min. after injection of a carrier-free solution of thorium-234 in 1 per cent sodium citrate at *p*H 5. The chelating agent was added to give a final concentration of $10^{-4}$ $M$.

It should be mentioned that, unlike the situation with radioactive metal poisons, where maximum elimination is a necessary goal, an effective therapeutic agent need not increase the ultrafilterability of a non-radioactive metal poison, and may in fact decrease it. A case in point is acute beryllium poisoning, in which successful treatment has been shown to involve inactivation of beryllium without necessarily increasing its excretion or diffusibility[10].

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# SAP-TRANSMISSIBLE VIRUSES ASSOCIATED WITH DISEASES OF GRAPE VINES IN EUROPE AND NORTH AMERICA

By C. H. CADMAN
Scottish Horticultural Research Institute, Invergowrie, by Dundee

H. F. DIAS* and B. D. HARRISON
Rothamsted Experimental Station, Harpenden, Hertfordshire

DEGENERATION of the grape vine has been recognized as a major factor in viticulture since the nineteenth century and has been attributed to many causes, ranging from unfavourable soil conditions to bacteria, fungi and viruses[1]. In Europe, three main groups of diseases have been transmitted by grafting and therefore ascribed to viruses[2]: (*a*) court-noué, Reisigkrankheit, urticado; (*b*) panachure, Panaschüre, clorose infecciosa; (*c*) enroulement, Rollkrankheit. Recently, Ochs[3] has claimed to have transmitted potato viruses *X* and *Y* and cucumber mosaic virus to herbaceous plants by inoculation of sap from grape plants affected by diseases of types (*a*) and (*b*). However, later work failed to confirm Ochs's results[4].

Virus diseases which appear similar to those in Europe also occur in Californian vineyards, and two of these diseases, fanleaf and yellow mosaic (similar to groups (*a*) and (*b*), respectively), are contracted from soil[5,6]. The virus that causes fanleaf disease is transmitted by a soil-inhabiting dagger nematode, *Xiphinema index* Thorne and Allen[7].

## Isolation of Viruses from European and North American Grape Vines

Sap from vine leaves seems to contain little or no tannin but has a *p*H value of about 3·5. Young grape leaves were therefore macerated in 0·07 *M* phosphate buffer at *p*H 8·0 or in 2·5 per cent nicotine solution, in an attempt to raise the *p*H value of the sap, and hence to lessen both virus inactivation and damage to inoculated leaves of test plants. 'Celite' abrasive powder was added to all inocula. Using this method, viruses were transmitted from diseased vines obtained from four different countries to *Chenopodium amaranticolor* Coste and Reyn., tobacco (*Nicotiana tabacum* L. var. White Burley), *N. clevelandii* Gray, cucumber (*Cucumis sativus* L.) and French bean (*Phaseolus vulgaris* L. var. Prince). No transmissions were obtained from normal plants of several of the same vine varieties (Table 1). The symptoms in *C. amaranticolor*, which resemble those caused by *Arabis* mosaic virus[14], are chlorotic lesions in the inoculated leaves and systemic symptoms, which start in the young leaves as chlorotic vein-banding or mottling; later, the whole plant is stunted and the leaves deformed. Different isolates from vine vary greatly in virulence, and systemic symptoms in *C. amaranticolor* take 7–20 days to appear, whereas those caused by *Arabis* mosaic virus usually take only 5–10 days.

In the other species symptoms also develop more slowly than with *Arabis* mosaic virus. All the vine isolates caused chlorotic or necrotic local lesions in inoculated leaves of tobacco and systemic mottling or necrotic ringspot symptoms in *N. clevelandii*. The Portuguese urticado *A*, Swiss court-noué and North American fanleaf *A* isolates produced systemic yellow blotching in cucumber, and chlorotic local lesions in inoculated leaves of French bean together with systemic mottling. Portuguese urticado *B*, Portuguese clorose infecciosa, French court-noué and North

Table 1. EXAMPLES OF VIRUS TRANSMISSIONS FROM GRAPE VINES TO HERBACEOUS SPECIES

| Country of origin | Variety* | Disease status | Grape vines supplied by | Transmission of virus to herbaceous species |
|---|---|---|---|---|
| Portugal | Fernão Pires | urticado *A* | H. F. Dias | + |
| Portugal | Fernão Pires | urticado *B* | H. F. Dias | + |
| Portugal | Fernão Pires | clorose infecciosa | H. F. Dias | + |
| Portugal | Fernão Pires | normal | H. F. Dias | 0 |
| France | Chardonnay | court-noué | A. Vuittenez | + |
| France | Vialla | panachure | A. Vuittenez | + |
| Switzerland | Chasselas | court-noué | R. Bovey | + |
| Switzerland | Chasselas | normal | R. Bovey | 0 |
| Switzerland | *Vitis* hybrid *S.* 5755 | panachure | R. Bovey | + |
| United States | *V. rupestris* St. George | fanleaf *A* | W. B. Hewitt | + |
| United States | *V. rupestris* St. George | fanleaf *B*† | W. B. Hewitt | + |
| United States | *V. rupestris* St. George | normal | W. B. Hewitt | 0 |

* *Vitis vinifera* unless otherwise stated.
† Vine plants to which virus was transmitted by *Xiphinema index* (ref. 7).

Grape vines are little grown in Britain; but three groups of soil-borne viruses have been reported in other crops[8], and members of each group also occur in Continental Europe[9–11]. All these viruses are readily transmitted by inoculation of sap to a wide range of herbaceous species, and one of them, *Arabis* mosaic virus, has a dagger nematode (*Xiphinema diversicaudatum* Micoletsky) as a vector[12,13]. We now report the transmission of viruses from grape vines to herbaceous hosts and describe some properties of the viruses. Our results show that viruses isolated either from North American or from European grape vines have similar properties to *Arabis* mosaic (= raspberry yellow dwarf) virus, and that they have some of their antigens in common with it.

* Calouste Gulbenkian Foundation Research Fellow from the Estação Agronomica Nacional, Portugal.

American fanleaf *B* isolates were less virulent than these three. Least virulent were French panachure and Swiss panachure isolates: these caused chlorotic local lesions in inoculated cucumber cotyledons but did not become systemic. They did not infect French bean, and they caused much milder symptoms in tobacco, *N. clevelandii*, and *C. amaranticolor* than those caused by the first group of isolates.

Many of the isolates (for example, Portuguese) came from grape plants that were infected by grafting, with ungrafted grape plants of the same clone serving as control material. The plants generously supplied from California by Dr. W. B. Hewitt, however, included six plants of *Vitis rupestris* var. St. George, each of which had become infected with vine fanleaf virus when exposed to infective *Xiphinema index*, and six normal St. George plants of the same clone. Virus was transmitted to *C. amaranticolor* and *N. clevelandii* from five fanleaf-infected plants (the sixth died) but from none of the normal ones, indicating that the isolates are consistently associated with fanleaf disease and are transmissible by *X. index*.

## Properties of the Viruses

Table 2 shows that the five isolates selected for more detailed study have broadly similar stabilities *in vitro*. The differences seem to result from the greater concentration in sap attained by the more virulent isolates.

Portuguese urticado *A* and Portuguese clorose infecciosa are examples of isolates that have similar effects on herbaceous plants and have similar properties *in vitro*, but yet come from grape plants that show very different types of symptoms: such isolates should not, therefore, be assumed to be strains of the same virus. However, they are sufficiently closely related for the presence of one to affect the other[15]. Thus, *C. amaranticolor* inoculated simultaneously with both isolates showed symptoms intermediate between those shown by plants infected with only one or other of the isolates. Similar interactions were observed between the Portuguese urticado *A* and a North American nematode-transmitted (fanleaf *B*) isolate.

Electron microscopy of partially purified preparations (made from *C. amaranticolor* by a butanol/chloroform method[16]) of the Portuguese urticado *A*, Portuguese clorose infecciosa and North American nematode-transmitted (fanleaf *B*) isolates showed that all three contain many spherical particles of diameter approximately 30 mμ (Fig. 1). No such particles occurred in preparations made by the same method from healthy *C. amaranticolor* plants.

Sap from *C. amaranticolor* plants infected with the vine isolates did not precipitate specifically with

Table 2. Properties *in vitro* of Virus Isolates from Grape Vines

| Isolate | Properties* | | |
|---|---|---|---|
| | Thermal inactivation point (°C.) | Dilution end-point | Longevity *in vitro* at 18° C. (days) |
| North American fanleaf *A* | 60–62 | 1/5,000–1/10,000 | 14–21 |
| Swiss court-noué | 60–62 | 1/1,000–1/2,500 | — |
| Portuguese urticado *A* | 63–65 | 1/5,000–1/10,000 | 21–28 |
| Portuguese clorose infecciosa | 58–60 | 1/500 –1/1,000 | 7–14 |
| French panachure | 58–61 | 1/500 –1/1,000 | 6–11 |

* In sap from *Chenopodium amaranticolor*. Figures are the limits between which infectivity was lost.

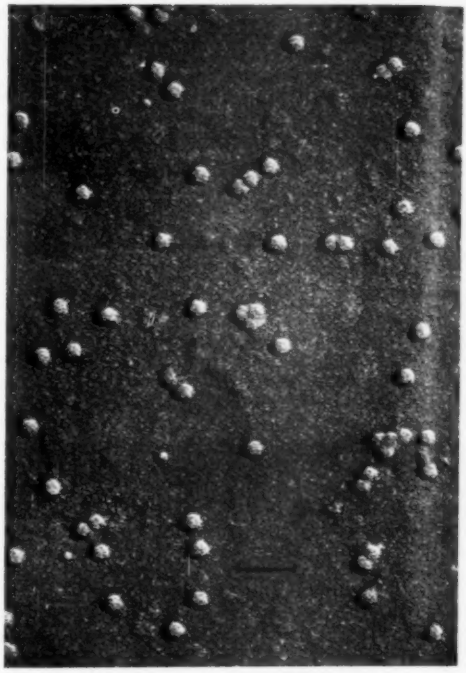

Fig. 1. Electron micrograph of a partially purified preparation of Portuguese urticado *A* isolate. Shadowcast with platinum/iridium. The scale mark represents 0·1μ

antisera to tomato black ring or raspberry ringspot viruses, two soil-borne viruses from Britain that have many properties in common with the vine viruses. By contrast, tests with antisera to *Arabis* mosaic virus and to North American fanleaf *A* isolate showed that these two viruses have some of their antigens in common (Table 3). All the French and Swiss isolates also precipitated specifically with both antisera, but at lower antiserum titres than the homologous viruses. There seems no possibility that the grape vine isolates were mixtures of *Arabis* mosaic and some other virus, for none of them infected *Petunia hybrida* plants systemically, a reaction characteristic of *Arabis* mosaic virus. Moreover, *C. amaranticolor* plants systemically infected with North American fanleaf *A*, Portuguese urticado, Swiss court-noué and French panachure isolates are susceptible to infection with *Arabis* mosaic virus. This is further evidence that the vine isolates and *Arabis* mosaic virus are only distantly related strains of the same virus.

## Association of *Xiphinema index* with Urticado Disease in Portuguese Vineyards

Although several workers have obtained evidence that vine viruses are contracted from the soil in Europe[17], and fanleaf virus is transmitted by *X. index* in California[7], the identity of the vectors in Europe is uncertain. In Portugal, urticado disease often occurs patchily in vineyards, and examination of soil samples from two different vineyards showed that the distribution of urticado coincides with that of a nematode probably identical with *X. index*, whereas another species of *Xiphinema*, *X. americanum* Cobb, occurred in soil from all parts of the vineyards. The

Table 3. PRECIPITATION END-POINTS OF ANTISERA TO *Arabis* MOSAIC AND GRAPE VINE VIRUSES

| Antigen* | Antiserum to† | | | | |
|---|---|---|---|---|---|
| | *Arabis* mosaic virus | *Arabis* mosaic virus (absorbed with 3 vol. fanleaf *B* antigen) | North American fanleaf isolate *A* | North American fanleaf isolate *A* (absorbed with 3 vol. *Arabis* mosaic virus antigen) | Normal rabbit serum |
| *Arabis* mosaic virus | 1/512 | 1/256 | 1/16 | 0 (1/4) | 0 (1/4) |
| North American fanleaf isolate *A* | 1/128 | — | 1/512 | 1/256 | 0 (1/4) |
| North American fanleaf isolate *B* | 1/128 | 0 (1/4) | 1/256 | — | — |
| Normal *Chenopodium amaranticolor* sap | 0 (1/2) | — | 0 (1/4) | — | 0 (1/4) |

* Partially purified preparations made from *C. amaranticolor* or *Nicotiana clevelandii* leaves and used at constant dilution.
† Figures are the precipitation end-points of the antisera after incubating with the antigens for 5 hr. at 35° C. Figures in parentheses are the smallest dilutions tested.

ecology of the virus causing urticado and other similar European vine diseases may thus be similar to that of vine fanleaf virus in California.

## Conclusions

Although it is not yet certain that the vine viruses can be transmitted to herbaceous plants so readily that this method can be used for routine diagnosis of virus infection in grape vines, its application on an experimental scale provides a new approach to the study of the relationship between the viruses associated with grape vine diseases, particularly those in the following two groups: (1) fanleaf, Reisigkrankheit, urticado, court-noué, and (2) yellow mosaic, clorose infecciosa, Panaschüre and panachure. Our results show that similar viruses can be isolated from plants with each of these diseases, regardless of the group to which the diseases belong, but not from normal plants. However, vines with 'yellow mosaic' may perhaps also contain avirulent strains of fanleaf virus, because 'fanleaf' is usually common in vineyards where 'yellow mosaic' occurs: thus, the isolates obtained from grape vines with 'yellow mosaic' may not be the cause of the yellow mosaic symptoms. We have transmitted North American fanleaf *A* isolate back to grape vine by grafting to infected *Chenopodium amaranticolor*, but to what extent the other viruses we have transmitted to herbaceous plants are responsible for the vine diseases with which they are associated must remain uncertain until further tests have been made. The results of the serological tests indicate that the sap-transmissible isolates from grape vines, and *Arabis* mosaic virus, are all strains of one virus, but they also suggest there are considerable antigenic differences between isolates. Further work is in progress to find whether these differences reflect vector specificity or geographical variation, or both.

We are much indebted to Dr. W. B. Hewitt, Davis, California; Dr. R. Bovey, Nyon, Switzerland; and Mr. A. Vuittenez, Colmar, France, for supplying normal and infected grape vines; to D. J. Hooper for identifying the nematode species, and to H. L. Nixon, J. Cathro and R. D. Woods for examining preparations with the electron microscope.

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# POLYPLOIDY IN HUMAN TUMOURS

By N. B. ATKIN and ANGELA J. ROSS
Department of Cancer Research, Mount Vernon Hospital, Northwood, Middlesex

MALIGNANT tumours, like normal tissues, exhibit varying degrees of polyploidy, but whereas in normal somatic tissues the great majority of cells usually retain the diploid chromosome complement, malignant tumours often have a pronounced secondary complement of polyploid cells, or may have undergone a shift to a basically near-tetraploid chromosome number. Although primary tumours may have an apparently normal chromosome complement, aneuploidy is a common finding and moreover is the rule in animal tumours which have been transplanted for a long time [1]; in the mouse, the change to aneuploidy often appears to involve a doubling of the chromosome complement, since many of the tumours have near-tetraploid chromosome numbers. The significance of polyploidy in the evolution of transplantable mouse tumours has been discussed by Levan[2,3], who has demonstrated that near-tetraploid tumours show much more evidence of structural chromosomal change than do near-diploid tumours. It is uncertain, however, to what extent the findings in experimental animal tumours have their parallel in human malignant disease.

We wish to present some evidence relating to the occurrence of polyploidy in human tumours. We have now accumulated data on the chromosome complement and deoxyribonucleic acid content of malignant tumours from a variety of different sites. Whereas extensive chromosome counts have been made on only a few suitable tumours, microspectrophotometric estimations of deoxyribonucleic acid, not being similarly restricted by technical considera-

tions, have been performed on a large number of tumours[4–6]. We have already noted[4] that, although individual tumours extend over a wide range, they tend to fall into two groups as regards their modal deoxyribonucleic acid value, which are centred in the hyperdiploid and tetraploid regions respectively. This has been confirmed by a further study on 165 uterine tumours[5], in which the deoxyribonucleic acid data have been considered in relation to the histological and clinical features of the tumours. In this work it was found that the cervical carcinomata (132 cases) fell into two approximately equal groups; the majority of corpus tumours however, fell into the hyperdiploid group. If the modal deoxyribonucleic acid value, derived from measurements on interphase cells, can be taken to be representative of the stem-line of the tumours, it would appear that, in a considerable proportion of cases, the stem-line chromosome complement of the tumour has doubled at some stage of its evolution.

It is of interest that published data on the chromosome numbers of human tumours do not appear to show a similar bimodal distribution[7–10]. This might be because there has been a selection of those tumours that prove most suitable for chromosome counting: near-triploid tumours may have been selected because they more frequently provide countable prometaphases or metaphases in squash preparations than do those having higher numbers. Another possible reason for the discrepancy is a lack of parallelism that we have found in some tumours between deoxyribonucleic acid content and chromosome number: in a series of 14 tumours[10] we found on an average a significantly higher ratio of modal deoxyribonucleic acid content to chromosome number than for the homologous normal cells, and it appeared that this difference was on the whole greater for those tumours with high chromosome numbers. Thus a nearly triploid tumour might have a modal deoxyribonucleic acid content which would correspond to a hypotetraploid chromosome number if the average amount of deoxyribonucleic acid per chromosome were the same as that in the normal diploid cells. Perhaps chromosomal doubling is commonly followed by structural changes and loss of chromosomal material, resulting in a reduction in the number of chromosomes and an increase in their average size.

It is of course possible that some of the tumours that are 'tetraploid' as regards modal deoxyribonucleic acid content are polytene and not polyploid, but we have so far obtained no direct evidence for the occurrence of polyteny in human tumours. It is also possible that the presence of a large component of cells which have synthesized deoxyribonucleic acid prior to mitosis might result in a tumour with a near-diploid stem-line chromosome number having an interphase population that is mainly 'tetraploid' as regards deoxyribonucleic acid content, as was in fact described for an ovarian carcinoma by Bader[11]. However, chromosome counts of greater or lesser accuracy on most of our 'tetraploid' tumours have been in keeping with the deoxyribonucleic acid data. Moreover, with a few exceptions (see below), the tetraploid tumours do not retain any cells having diploid amounts of deoxyribonucleic acid, and where measurements of deoxyribonucleic acid have been made on cells in mitosis, these have been consistent with a near-tetraploid rather than a near-diploid chromosome number.

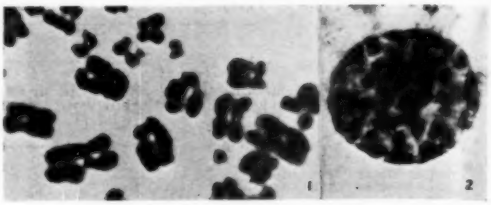

Fig. 1. Endoreduplication: diplochromosomes at metaphase (carcinoma of the corpus uteri). (× 2700)
Fig. 2. Endomitosis (carcinoma of the cervix uteri). (× 1320)
Acetic orcein squash preparations

Our results are derived from patients at the time that they enter hospital for treatment, and it is not known at what stage the doubling of the chromosome complement has occurred in the near-tetraploid tumours. Evidence is forthcoming, however, from cytological studies that polyploidization is occurring in some of these tumours. The work of Biesele, Poyner and Painter[12] and of Levan and Hauschka[13] has demonstrated the part played by endomitosis and endoreduplication in the growth of experimental tumours of the mouse. In human tumours, we have likewise noted these two phenomena; but whereas endoreduplication, as revealed by the presence of diplochromosomes in succeeding mitoses, has been found occasionally in a number of tumours, we have noted that certain tumours are characterized by a high frequency of cells exhibiting chromosomal contraction and splitting within the nuclear membrane, corresponding to the classical picture of endomitosis[14]. The highest incidence of endomitosis was seen in certain undifferentiated squamous cell carcinomata and adenoacathomata of the cervix uteri. In general, endomitosis was a fairly frequent phenomenon in squamous cell tumours, but was only rarely seen in adenocarcinomata. The deoxyribonucleic acid data derived from tumours showing a high frequency of endomitosis are of interest: the modal deoxyribonucleic acid value fell most often in the hypertetraploid region, but several tumours also had a smaller secondary hyperdiploid mode; a few of the tumours had a hyperdiploid primary mode and a prominent secondary hypertetraploid mode. These tumours may be in a state of equilibrium; alternatively, they may be in the process of transition from a hyperdiploid to a hypertetraploid state. It was noted that endomitosis was not a feature of tumours which had a hypotetraploid deoxyribonucleic acid mode.

The question arises as to whether the incidence of endomitosis and endoreduplication in a particular tumour is an unchanging feature of the tumour, or whether it is dependent on environmental influences, for example, varying hormonal levels. In Levan and Hauschka's[13] work on mouse tumours, each tumour was characterized by a particular level of endomitosis or endoreduplication. In certain normal tissues, however, there is evidence that hormonal factors may determine the occurrence of polyploidy. Thus Arias-Stella[15] has demonstrated the association of nuclear hypertrophy in endometrium with retained chorionic tissue. He has suggested that the atypical pattern results from hormonal activity of the chorionic tissue, and also quotes instances of nuclear enlargement in other tissues which may be the result of endocrine activity. The occurrence of polyploidy in human[16] and mouse[17] liver may be dependent on anterior pituitary growth hormone. So far as the

human cervical tumours are concerned, no clear evidence for a hormonal or other 'host' factor has come to light, but an apparent difference in age-distribution between tumours with different deoxyribonucleic acid modes is perhaps suggestive of a hormonal influence: it was noted[5] that the age-distribution of those tumours with high modal deoxyribonucleic acid values was flatter, with relatively fewer cases in the middle age-groups, than that of the near-diploid tumours, and 11 cases aged over 74 had tetraploid modes, as compared with one which was diploid. These differences, however, are not statistically significant. On the other hand, when we calculate the relative height of the secondary (tetraploid) mode in 25 near-diploid squamous cell cervical tumours chosen at random, we find that in 9 cases more than 20 per cent of the cells fall within $\pm$ 15 per cent of the secondary mode, and of these 7 were from patients aged over 54. Only 5 of the 16 tumours with smaller secondary modes were from patients aged over 54. This difference is just significant ($p = 0{\cdot}033$). A similar calculation for 18 near-tetraploid tumours shows that 6 out of 8 occurring in patients aged over 54 have prominent secondary octoploid modes, as compared with 4 out of 10 in younger patients. The presence of a prominent secondary mode at twice the value of the primary mode does not necessarily imply that there are many cells with a doubled chromosome complement; but there is no evidence that other factors, such as the frequency of cells entering mitosis, are responsible for the observed differences, which may therefore reflect the incidence of endomitosis or other doubling processes.

It was found[5] that the modal deoxyribonucleic acid value of nearly a third of the squamous cell tumours of the cervix fell within $\pm$ 15 per cent of the normal diploid value. Most of these tumours had only small or moderate secondary 'tetraploid' modes. The tumours showing endomitosis, however, frequently had modes (primary and secondary) which were at least 15 per cent higher than the corresponding normal diploid or tetraploid values. It is hoped that further work now being undertaken will shed some light on the significance of this difference and on other problems connected with the occurrence of endomitosis and related phenomena in human tumours. As to the relation between polyploidy and the degree of differentiation of the tumours, we have noted that there appears to be a correlation in the case of tumours of the corpus uteri, well-differentiated tumours being mainly near-diploid; no such correlation has, however, been found for squamous cell carcinomas of the cervix, or for squamous cell tumours arising at other sites.

In summary, human tumours frequently have a primary modal deoxyribonucleic acid value in the tetraploid region, suggesting that chromosomal doubling has occurred in the majority of, or all, the cells. Further, cytological observations suggest that endomitosis or some other doubling mechanism may play a part in the growth of human tumours, endomitosis in particular being a prominent feature of some tumours, which usually have hypertetraploid modes. There is evidence to suggest that the incidence of polyploidy in carcinoma of the cervix may bear a relation to the age of the patient.

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# THE ANTARCTIC CONVERGENCE—AND DIVERGENCE

By Dr. K. WYRTKI

Division of Fisheries and Oceanography, Commonwealth Scientific and Industrial Research Organization, Cronulla, Sydney

IN a recent paper, Wexler[1] gives observational evidence of prevailing divergent motion along the line which is usually called the Antarctic Convergence. Mackintosh[2] has determined the position of this line from surface-temperature data, considering the strong temperature gradient at the boundary of the antarctic and the sub-antarctic surface waters as indicative of its position. But until now no observational evidence has been given for convergent motion along this line, as the sinking of the antarctic intermediate water occurs usually a few degrees farther to the north, as can be seen from the salinity sections given by Deacon[3].

Considering the Antarctic Convergence as a surface phenomenon in the light of Ekman's theory, it is evident that divergent motions connected with upwelling must occur to the south of the maximal westerlies and convergent motions and sinking to the north of them, but such a transverse circulation would not lead to the formation of a distinguishable water mass boundary. Consequently, this boundary must be related to the dynamics of the antarctic circumpolar current. Stommel[4] has sketched a Sverdrup-type solution for the circumpolar current, in which the wind stress is balanced by a zonal pressure gradient, caused by the boundary formed by South America. This model explains the southward shift of the current; but it implies that the current is situated completely to the south of the maximal westerlies.

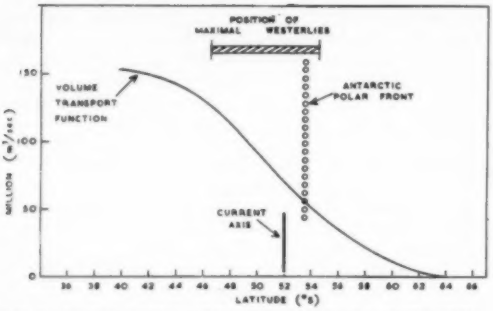


Fig. 1 Average positions of the belt of maximal westerlies of the antarctic polar front and volume transport function of the antarctic circumpolar current

In order to check the relative positions of the belt of maximal westerlies, the circumpolar current and the Antarctic Convergence, their average positions have been determined. In Fig. 1 the position of the belt of maximal westerlies according to Wexler[1], that of the Antarctic Convergence according to Mackintosh[2] and the volume transport function relative to the antarctic continent according to Kort[5] are given. This diagram shows clearly that the axis of the circumpolar current coincides almost with the maximal westerlies and that the Antarctic Convergence lies just to the south of this position. Consequently the circumpolar current cannot be explained exclusively by a Sverdrup-type solution; but friction must also be of importance, and its effect will be to make the current coincide with the west wind belt. In fact, in Wexler's diagram, this belt shows a shift to the south, corresponding to that of the circumpolar current.

A frictional conception of the circumpolar current allows an explanation of the water mass boundary, usually called the Antarctic Convergence. In the presence of friction the motion is no longer geostrophic, but has a down-slope component. If friction is considered to be proportional to the velocity, the angle $\alpha$ between the velocity vector and the isobars is given by $\tan \alpha = \frac{r}{f}$, where $f$ is the Coriolis parameter and $r$ the friction coefficient. With $f = 10^{-4}$ sec.$^{-1}$ and $r = 2\ 10^{-6}$ sec.$^{-1}$, $\tan \alpha$ is 1/50 and $\alpha = 1{\cdot}2°$, which is indeed a small angle.

The meridional motion at the surface near the centre of the current is now the result of the combined effects of the northward component of the Ekman drift current and of the down-slope motion due to friction, caused by the meridional pressure gradient. The north component of the velocity $v_E$ of the Ekman drift current is $v_E = \frac{\pi \tau}{\delta D f}$, where $\tau$ is the zonal wind stress and $D$ the depth of the Ekman layer. With $\tau = 0{\cdot}8g$ cm.$^{-1}$ sec.$^{-2}$, $D = 100$ m., $\delta = 1g$ cm.$^{-3}$, $v_E = 2{\cdot}5$ cm./sec. results, representing the north component of the drift current under average wind conditions. The down-slope component of the gradient current, on the other hand, is given by $v_g = \frac{1}{r}\frac{\partial h}{\partial y}$, where $\frac{\partial h}{\partial y}$ is the meridional slope of the sea-level. Across the current axis a slope of 50 cm. within 100 km. is quite a likely value, and at the above given value of $r$, a velocity of 2·5 cm./sec. would result in a southward direction.

With these estimates of the meridional components of the motion near the centre of the current, we can now discuss the effect of strong and of weak winds on the transverse circulation in the current. At strong west winds ($\tau > 0{\cdot}8$) the surface flow is divergent to the south of the maximal westerlies, a northward transport of surface waters occurs across the current axis, because the velocity of the drift current exceeds the down-slope velocity, and convergent motion occurs to the north of the maximal westerlies. This pattern of the transverse circulation is sketched in Fig. 2*A* and is almost identical with the conception of Wexler[1] of divergent and ascending motion just to the south of the maximal westerlies. At weak west winds ($\tau < 0{\cdot}8$) the down-slope flow in the centre of the current exceeds the drift current, causing a convergence at the right flank of the main current and a sinking motion, which intensify the water mass boundary there. The circulation pattern (Fig. 2*B*) shows, moreover, a divergence to the north of the maximal westerlies, which appears clearly in charts of surface currents[6].

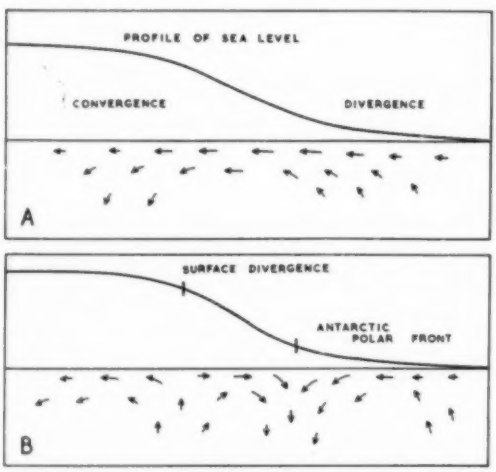


Fig. 2. Schematic representation of the topography of sea-level and of the transverse circulation in the surface layer of the antarctic circumpolar current at (*A*) strong west winds, and (*B*) weak west winds

A diagram giving G.E.K. vectors along a section across the circumpolar current, taken by a Russian ship[7] along 20° E., shows clearly that the meridional components of the motion are southwards between 49° and 51° S., where the temperature diagram indicates the Antarctic Convergence, and northwards to the north as well as to the south of this belt.

From this discussion it must follow that the 'Antarctic Convergence' is a divergence at strong west winds, and a convergence at weak west winds, but as it is always a water mass boundary and a clearly distinguishable feature in the antarctic waters, it is suggested that it be named the 'Antarctic Polar Front'. This does not specify the character of vertical movements along this line and is in accordance with the terminology used for the corresponding feature in the northern hemisphere.

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# LETTERS TO THE EDITORS

## COSMOLOGY

### Interpretation of Cosmology

PROF. W. H. MCCREA'S views on the interpretation of cosmology[1] call for some comment. If the universe turns out to have an inhomogeneous character, then his remarks may be justified. But, in view of the paucity of observational tests that have been adequately investigated so far, it would seem that he takes up a prematurely pessimistic position.

McCrea asserts that there is an uncertainty of knowledge of the factors affecting the physical conditions in those remote parts of the universe which we now see at an early stage in their history, due to the finite velocity of light. This uncertainty, he claims, increases with the observed red shift, and he is led to suggest that differences between various cosmological models cannot be detected as a matter of principle. In particular, calculated distinctions between a steady-state universe and an evolving universe may be meaningless.

There is, of course, uncertainty about the factors affecting any set of phenomena, until we put forward a definite theory accounting for them. For this purpose it may even be necessary to introduce factors or agents that we cannot ourselves observe, if we have reason for believing that they must be observable to other observers whose past experience has been similar to our own. This is in accordance with accepted scientific method. We must try to fit the facts to what seems the most likely interpretation.

In the case of the cosmological problem the simplest and most direct approach, suggested by the Hubble law and the nebular counts, is to assume the 'cosmological principle'. This leads to a definite set of cosmological models: one is the steady-state model and the rest are evolutionary.

As I have shown in recent papers[2,3] there are several tests which, given a plausible continuation of present advances in astronomical technique, should definitely decide whether the universe is in a steady state or not. It is true, as McCrea points out, that the steady-state predictions differ from those of the evolutionary cases only in terms of order $z$, where $z$ is the red shift. But on the other hand, the predictions for a steady-state model are quite definite and must be realized in a steady-state universe on a statistical basis, if we always choose fair samples for our observational data. There is no uncertainty in principle about the conditions prevailing in remote regions of a steady-state universe, since they must be the same conditions that we experience in our own 'neighbourhood' at the present moment. Consistent agreement of the universe with the various features predicted by all possible tests for the steady-state model must therefore establish it, scientifically, as the correct fit. Consistent disagreement, in ways that cannot be explained by observational errors, must eliminate it.

If the universe is found to be not in a steady state then, retaining the cosmological principle, we must next regard it as evolving in a systematic way. In this case there are indeed great difficulties in predicting exactly what we should see in remote regions in the various models, because of the uncertain degree of evolution that has taken place during the 'light time'. However, it should be appreciated that, by the cosmological principle, the conditions viewed right up to our observational horizon were realized in our own neighbourhood at the same cosmic epochs in the past. It is not inconceivable, therefore, that we may ultimately find an adequate description of these phenomena in agreement with our own discovered past. Such a possibility should not be excluded as a matter of principle.

The difficulties of interpretation which arise in an evolutionary universe were of course pointed out by the original advocates of the steady-state model[4]. Even greater difficulties arise if we drop the cosmological principle and assume, say, a hierarchical model of the universe. In this case the uncertainty of the universe *in toto* would indeed be of the fundamental character that McCrea has envisaged.

An observational decision in favour of a steady-state, evolutionary, or hierarchical universe would have an importance not confined to physics. In due time it would find its place in the basic philosophical outlook of all mankind. Every possible effort should therefore be made to reach it.

W. DAVIDSON

Mathematics Department,
Battersea College of Technology,
London, S.W.11. June 30.

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DR. DAVIDSON re-states the orthodox view, and it is this view that I venture to call in question.

Suppose we know all about the laws of interaction of different parts of the universe, and suppose we make all the observations of the universe that are possible to us in principle. Then, for the reasons that I stated, I conclude that there is an uncertainty about what we can predict about the universe. The orthodox view is that theory can enable us to overcome this uncertainty. The view I suggested is that we cannot do better than in the circumstances just supposed, and that therefore theory ought to be modified so that it does not claim to do better.

The uncertainty contemplated here is of a more novel and fundamental kind than appears to be altogether recognized in Dr. Davidson's discussion. Also, there is nothing necessarily pessimistic about it. If we could properly take account of it, it would be expected to lead to the discovery of new phenomena just as the sacrifices made in going from classical theory to relativity and quantum theory led to the discovery of new phenomena. Finally, there is nothing contrary to the 'accepted scientific method'. For, in regard to what Dr. Davidson has to say on the subject, we have to recall that no amount of agreement with observation can 'establish' a theory; all that such agreement means is the absence of what we deem to be a contradiction.

W. H. MCCREA

Royal Holloway College,
Englefield Green, Surrey.

## PHYSICS

### Diamagnetic Moment of Gaseous Plasmas measured by Spin Resonance Techniques

We report here typical experimental results obtained in measuring the diamagnetic moment of plasmas immersed in static or quasi-static magnetic fields. Nuclear and electron spin resonance techniques were used for detection of magnetic field shifts; the results indicate that such techniques may be of value as a diagnostic tool in high-energy plasma research.

A well-known theorem in statistical mechanics by Van Leeuwen[1] states that the magnetic moment of a classical free electron gas in thermodynamic equilibrium in a magnetic field is zero. The physical interpretation of this result is due to N. Bohr[2], who argued that the moment due to the electron Larmor orbits in the volume of a substance is identically cancelled by a boundary current of electrons elastically reflected by its surface.

Since Bohr considered the electron gas in a metal, which is degenerate rather than classical, his result appears to be irrelevant. Also, in view of the larger paramagnetic susceptibility of the electron gas in most metals, the diamagnetic moment from the Larmor motion is inaccessible to direct measurement.

A dense free electron gas in a laboratory gaseous plasma is a classical gas which ordinarily is Maxwellian distributed except under special conditions. However, in general it is not in thermodynamic equilibrium; energy and particles are diffusing continuously to the surface or other limiting boundaries. The bounding surface, which provides a region of recombination for electrons and ions in a plasma, is more absorbing than reflecting. It follows that a gaseous plasma immersed in a magnetic field will have a non-zero diamagnetic moment. Using a conventional model for ambipolar diffusion in a plasma, Alfven[3] showed that the moment of the plasma should be:

$$M = -\frac{nk(T_e + T_i)}{H_0} \frac{1}{1 + \frac{\nu_e \nu_i}{\omega_{He}\omega_{Hi}} + \frac{\nu_e^2}{\omega_{He}^2} + \dots} \quad (1)$$

where $n$ is the number density of charges, $T_e$ and $T_i$ are the electronic and ionic temperatures, $\omega_{He}$, $\omega_{Hi}$ are the electronic and ionic cyclotron frequencies, and $\nu_e$, $\nu_i$ are the collision frequencies for momentum transfer of the electrons and ions. For our work at low pressures and degrees of ionization (less than 3 per cent) and relatively high electron temperatures (greater than $10^4$ deg. K.), the momentum transfer collisions are determined by the elastic and inelastic cross-sections of the neutral molecules. $M$ is a maximum for $\nu_e\nu_i = \omega_{He}\omega_{Hi}$.

To measure the functional form of $M$ for low fields, we use an electronic spin resonance in diphenylpicrylhydrazyl[4] to record the small decrease in magnetic field observed just outside the discharge tube on its axis. This is done by placing about 0·1 gm. of diphenylpicrylhydrazyl in a coil which forms one arm of a balanced bridge, fed by a radio-frequency of 40–160 Mc./s. Resonances about 1½ gauss wide are displayed on an oscilloscope when $H_0$ is modulated by a periodic signal. If the plasma is pulsed every other period, then the entire resonance is displaced alternately on the oscilloscope, and field shifts as low as 0·1 gauss may be detected. The results of such a study on a low-pressure d.c. mercury discharge are shown in Fig. 1. The moment, which is obviously zero at zero field, appears to reach a maximum near 50 gauss, and from equation 1, one obtains an energy density of 2·8 ergs/cm.³. This agrees within 10 per cent with data on energy density taken by pulsed Langmuir probe techniques[5]. Low-field measurements on $M$ taken many years ago by Steenbeck[6], using different methods, agree with our results.

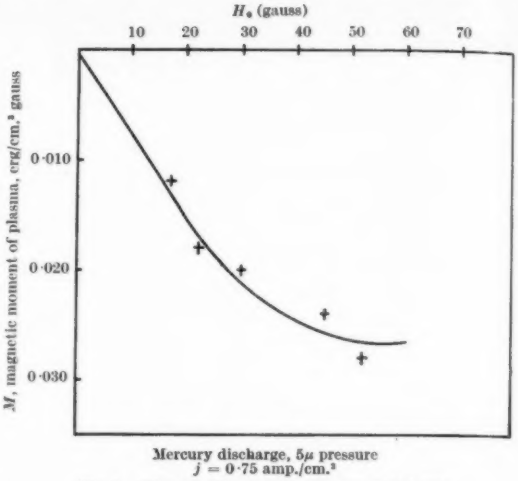


Fig. 1. Diamagnetic moment of a mercury plasma

The above method is limited in its applications by (1) poor sensitivity because of the wide resonance line width of diphenylpicrylhydrazyl and (2) because measurements of the shift of the electronic spin resonance must be made in the presence of a noisy plasma.

For measurements at high magnetic field intensities, we have used a '$\pi/2$ pulse' proton resonance technique such as described by Hahn[7] and Spokas and Slichter[8]. A pulse of radio frequency, tuned to the resonance frequency of protons in a glycerine sample in or near a plasma in a Varian electromagnet, nutates the nuclear magnetization 90 degrees into a plane perpendicular to $H_0$ in a few microseconds. The radio-frequency excitation is removed from the sample, and the frequency of precession of the nuclear spin magnets following the pulse is compared with the frequency of the radio-frequency source; the accumulated phase difference between the two signals provides a measure of the field deviation from resonance. Any

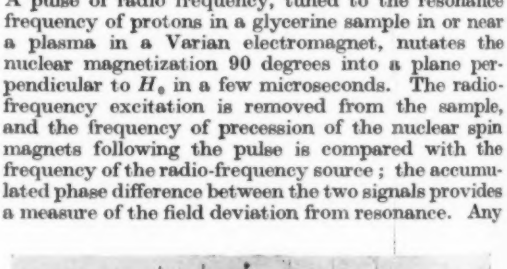

Fig. 2. Upper traces, nuclear magnetic resonance signals for phase comparison; lower trace, discharge tube current. 200 $\mu$sec./cm.

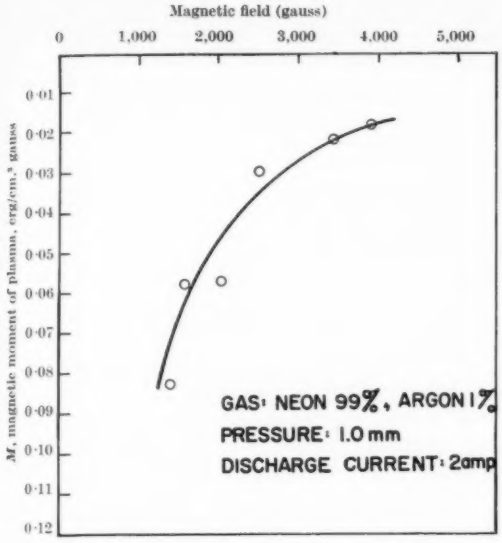


Fig. 3. Diamagnetic moment of a noble gas plasma

field produced by the diamagnetism of the plasma will further alter this deviation.

Fig. 2 shows the signals obtained. The upper damped sinusoid shows the nuclear resonance signal as it is phase detected after removal of the radio-frequency pulse (over-loaded portion far to left). The lower trace shows the same signal, but after a discharge current pulse was applied to a xenon plasma at about 5$\mu$ pressure. The discharge tube current, about 6 amp., is displayed as the square pulse at the lower left. The spin precession signals are displaced in phase and in this instance the field shift amounts to 0·12 gauss. The discharge tube here was a modified Phillips ion gauge type[9] which gives a high ionization efficiency in a magnetic field. The energy density is about 50 ergs/cm.$^3$. The phase shift was consistent in sign with the diamagnetism. Stray fields owing to currents passing through the discharge tube were found to give no observable phase shifts of their own. Fig. 3 shows typical results obtained in a relatively strong magnetic field.

The limit of small field detection would appear to be about 1 part in $10^6$, with suitable narrow-banding techniques to improve the signal/noise ratio. Note that these measurements can be made after the plasma discharge, the principal noise source, is over.

An experiment to increase further the electronic energies by cyclotron resonance heating of the electrons at microwave frequencies is now in progress.

The above results imply that spin resonance techniques may be useful in high-energy plasma research and thermonuclear work, for example: (1) Measuring energy densities of plasmas in magnetic fields utilizing plasma diamagnetism as given by equation 1. (2) Measuring any quasi-static magnetic field in a discharge using a small nuclear magnetic resonance probe, which yields $\int B(t)\,dt$ directly.

We gratefully acknowledge the assistance of Prof. C. P. Slichter in giving advice on the nuclear resonance experiment.

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T. C. Marshall
R. A. Kawcyn
L. Goldstein

University of Illinois,
Urbana,
Illinois.

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## Distorted Wave Approximation for Scattering Phase-Shifts

The elastic scattering wave-function for a short-range potential function $V(r)$ may be written in the form:

$$\psi_l(r) = kr[j_l(kr)\cos\delta_l - g_l(r)n_l(kr)\sin\delta_l] \qquad (1)$$

where $j_l(x)$ and $n_l(x)$ are the spherical Bessel and Neumann functions respectively, $\delta_l$ is the scattering phase-shift, and $g_l(r)$ is a form factor measuring the distortion of the incident wave by the potential. $g_l(r)$ must satisfy the boundary conditions

$$g_l(r \to 0) \sim r^{2l+1},\ g_l(r \to \infty) \to 1$$

We substitute (1) into the integral equation for the phase-shift[1]:

$$\sin\delta_l = -(2\mu\hbar^2k)\int_0^\infty V(r)kr j_l(kr)\psi_l(r)dr \qquad (2)$$

leading to:

$$\tan\delta_l = B_{1l}/(1 - B_{2l}) \qquad (3)$$

where:

$$B_{1l} = -(2\mu/\hbar^2k)\int_0^\infty V(r)k^2r^2j_l^2(kr)dr \qquad (4)$$

$$B_{2l} = (2\mu/\hbar^2k)\int_0^\infty g_l(r)V(r)k^2r^2j_l(kr)n_l(kr)dr \qquad (5)$$

$B_{1l}$ is the first Born approximation integral and $B_{2l}$ a new integral which is large at low energies, corresponding to strong distortion of the incident wave-function, and small at high energies.

The simplest choice of $g_l(r)$ is made by introducing a cut-off range $r_a$ beyond which the potential tail is negligible:

$$\begin{aligned} g_l(r) &= f_l(r) \quad (r \leqslant r_a) \\ &= 1 \quad (r \geqslant r_a) \end{aligned} \qquad (6)$$

$f_l(r)$ must obey the boundary conditions:

$$f_l(r \to 0) \sim r^{2l+1},\quad f_l(r_a) = 1,\quad f_l'(r_a) = 0 \qquad (7)$$

$g_l(r)$ and $f_l(r)$ are in general series expansions in $r$. However, $g_l(r)$ is involved only in the integral (5) for $B_{2l}$, and this has an integrand behaving like $r^{2l+2}$ for small $r$. Also for $r > r_a$, this integrand is negligible, so that $B_{2l}$ is determined almost entirely

by intermediate values of $r$ which are less than $r_a$. For light nuclei and small and intermediate $k$ values, $f_l(r)$ may be approximated by any monotonically increasing function of $r$ satisfying the constraints (7). To a first approximation, $B_{2l}$ is independent of the detailed shape of $f_l(r)$ as the latter's contribution to the integral is dependent mainly on the second and third conditions (7). Thus $B_{2l}$ is a function of $r_a$ alone to a first approximation.

For non-singular potential wells, $B_{2l}$ is a slowly varying function of $r_a$, so that $r_a$ may be chosen arbitrarily by inspection of the potential. Singular wells such as the Yukawa potential are somewhat more sensitive to the choice of $r_a$. In general, best results are obtained if the zero-energy scattering length $a$ is known for the potential $V(r)$. Using the definition :

$$a = \lim_{k \to 0}\left(-\frac{\tan\delta_0}{k}\right) \qquad (8)$$

(3) may be used to find the value of $r_a$ leading to the correct $a$. Values of $r_a$ obtained by this zero energy normalization procedure are slightly different according to the function used for $f_l(r)$. A simple polynomial expression which is found to give good results for a number of potential shapes is :

$$f_l(r) = \tfrac{1}{2}[(2l+3)(r/r_a)^{2l+1} - (2l+1)(r/r_a)^{2l+3}] \quad (9)$$

For $k$ large, $B_{2l}/B_{1l} \to 0$ and $\delta_l$ is small, so we get a second Born approximation from (3) :

$$\delta_l \approx B_{1l}(1 + B_{2l}) \qquad (10)$$

For $k$ small, (3) reduces to the shape-independent formula[2] :

$$k\cot\delta_0 = 1/a + \tfrac{1}{2}r_0k^2 + 0(k^4) \qquad (11)$$

with approximate values of the parameters $a$ and $r_0$.

A simple illustration of the application of formula (3) for $\delta_l$ is given by the $^3S$ and $^1S$ scattering of neutrons by protons at low energies, assuming square well potentials. We take for the nuclear well dimensions the following values[3] :

$^3S$ : $V_0 = 36{\cdot}354$ MeV., $b = 2{\cdot}0048 \times 10^{-13}$ cm.

$^1S$ : $V_0 = 14{\cdot}052$ MeV., $b = 2{\cdot}5860 \times 10^{-13}$ cm.

where $V_0$ and $b$ are the depth and range respectively of the potential.

In this case we should take $r_a = b$. Values of $\delta_0$ calculated by the exact formula, by the first Born approximation $B_{10}$, and by (3) are given in Table 1.

Table 1. Calculated Values of Phase-Shifts $\delta_0$ for $^3S$ and $^1S$ $n$–$p$ Scattering by Square Wells

| Spin state | $k$ | Exact $\delta_0$ | Born approximation $B_{10}$ | $\delta_0$ from (3) |
|---|---|---|---|---|
| $^3S$ | 0·1 | 2·6283 | 0·2337 | 2·6019 |
| | 0·25 | 2·0590 | 0·5600 | 2·0381 |
| | 0·5 | 1·5176 | 0·9626 | 1·5319 |
| $^1S$ | 0·1 | 1·0626 | 0·1928 | 1·0298 |
| | 0·25 | 1·1020 | 0·4493 | 1·1055 |
| | 0·5 | 0·9214 | 0·6979 | 0·9479 |

Values of $\delta_0$ are improved by an order of magnitude at all energies over first Born approximation results. Similar results are obtained for other potential shapes with $r_a$ chosen in these cases by the zero energy normalization method above.

For non-singular wells, such as gaussian, exponential or Woods–Saxon interactions, the accuracy of phase-shifts obtained using (3) is better than 2 per cent. Singular wells such as the Yukawa potential may give somewhat less accurate (but still good) results, as the spherical Neumann function $n_l(kr)$ in (1) no longer correctly describes the irregular solution of the wave equation. This actually contains logarithmic terms for a potential with a $1/r$ singularity.

P. Swan

Physics Department,
University of Melbourne.

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## METEOROLOGY

### Small-Scale Turbulent Diffusion in the Atmosphere

Frenkiel and Katz[1] have recently published under this title the results of their experiments with instantaneously created smoke puffs. Starting from Taylor's expression for the variance of the mean concentration in such a puff, assuming $R(\alpha)$, the Lagrangian correlation function, to be near unity for small values of the diffusion time, they have derived an expression for the radius of the outer visible boundary of the smoke puff, $a$, as a function of the diffusion time, $t$. They arrived at a nearly linear relationship between $a$ and $t$ for small values of $t$. This conclusion is fully supported by their experimental results.

However, one can doubt the validity of the assumption $R(\alpha) \sim 1$, since the experiments were carried out under conditions of unstable equilibrium of the atmosphere. According to Sutton's arguments[2], or following the more rigorous treatment of Clarenburg[3] for the diffusion of smoke puffs emerging from an instantaneous point source, it seems better to put $a$ proportional to $t^{2-n/2}$, at least for small values of $t$, where $n$ is a parameter depending on the turbulent state of the atmosphere.

To decide which of the two relations is the better one, we have calculated : (1) $R(a,t)$, the correlation of $a$ as a function of the time and the diffusion time plus 3 sec., thus allowing for the fact that the experiments were not carried out with a perfect point source. (2) $R(a,t^{2-n/2})$, the correlation of $a$ as a function of the time and $(t+3)^{2-n/2}$.

The results obtained are summarized in Table 1. The values of $a$ as a function of the time were taken from the tables published by Frenkiel and Katz.

Table 1

| Date | Puff No. | $R(a,t)$ | $R(a,t^{2-n/2})$* | $R(a,t)-R(a,t^{2-n/2})$ |
|---|---|---|---|---|
| 27/2/1950 | 4 | 0·9937 | 0·9948 | − 0·0011 |
| | 5 | 0·9644 | 0·9670 | − 0·0026 |
| | 7 | 0·9442 | 0·9464 | − 0·0022 |
| | 8 | 0·9563 | 0·9599 | − 0·0037 |
| | 16 | 0·9872 | 0·9862 | + 0·0010 |
| | 18 | 0·9813 | 0·9894 | − 0·0081 |
| | 19 | 0·9642 | 0·9644 | − 0·0002 |
| | 20 | 0·9784 | 0·9808 | − 0·0024 |
| 28/2/1950 | 1a | 0·9865 | 1·0000 | − 0·0135 |
| | 1b | 0·9554 | 0·9610 | − 0·0056 |
| | 2a | 0·9842 | 0·9851 | − 0·0009 |
| | 2b | 0·9933 | 0·9941 | − 0·0008 |
| | 3 | 0·9909 | 0·9899 | + 0·0010 |
| | 5 | 0·9634 | 0·9668 | − 0·0034 |
| | 9 | 0·9841 | 0·9821 | + 0·0020 |
| | 10 | 0·9650 | 0·9698 | − 0·0048 |
| | 11 | 0·9760 | 0·9846 | − 0·0086 |

*For $n$ the value 0·26 was taken.

As can be seen from Table 1, both correlations are highly significant. However, $R(a,t^{2-n/2})$ is in nearly all cases somewhat greater than $R(a,t)$. Applying Student's $t$ test to the differences $[R(a,t) - R(a,t^{2-n/2})]$, it appears that $R(a,t^{2-n/2})$ is significantly ($P < 0{\cdot}5$ per cent) higher than $R(a,t)$.

From the experiments of Frenkiel and Katz it may therefore be concluded that the more complete theory of diffusion yields results which are in better agreement with the experimental data. Because the difference between our treatment and that of Frenkiel and Katz is already clearly demonstrated for unstable atmospheric equilibrium, it must be expected, for reasons the discussion of which would be beyond the scope of this communication, that this difference will be even more pronounced for stability of the atmosphere.

We wish to thank the Board of the National Defence Research Council, T.N.O., for permission to publish this communication.

L. A. Clarenburg
C. E. Spaans-v. d. Tang

Chemisch Laboratorium RVO-TNO,
Rijswijk (Z.H.), Nederland.

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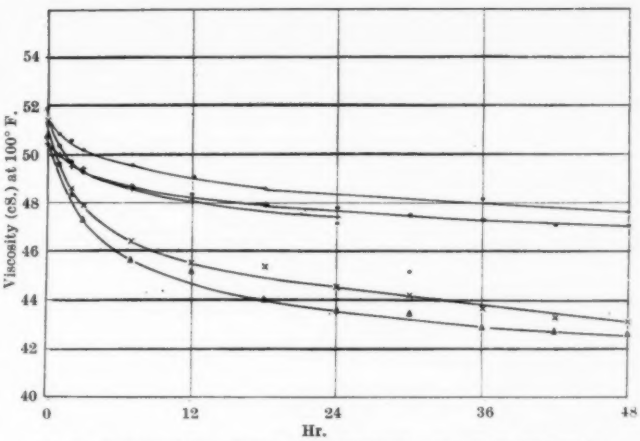


Fig. 1. Permanent loss of viscosity of lubricant. ●, Engine 'motored'; ○, lead-free fuel; +, fuel containing 0·21 ml. tetraethyl lead/Imp. gallon; ×, fuel containing 1·85 ml. tetraethyl lead/Imp. gallon; △, fuel containing 3·57 ml. tetraethyl lead/Imp. gallon

## ENGINEERING

### Initiation of Chemical Reactions by Mechanical Forces

It is well known that large molecules can be degraded mechanically, and the rupture of a chemical bond by a high shearing stress under conditions of laminar flow has been demonstrated with a 'jet viscometer'[1]. The free radicals formed on degradation by shear give rise to oxidation and other chemical reactions. Evidence of such an initiation of chemical reactions by mechanical forces was found with lubricating oils which had been used in an internal combustion engine while the engine was run 'cool' (sump temperature: 69·4° C.). A comparison was made of the change in viscosity and the dependence of viscosity on temperature of the oils when, on one hand, the engine was driven by an electric motor and, on the other hand, run on a fuel the lead content of which was varied. It appeared that the lead content of the fuel promoted a breakdown of large molecules and the combination of free radicals with oxygen molecules, which in their turn formed linkages for the growth of splinter molecules.

In all these experiments the viscosity of the oil decreased logarithmically with the time of running the engine, while the temperature coefficient of the viscosity increased. The permanent loss of viscosity during running of the engine on a lead-free fuel was very similar to that obtained when the engine had been driven by an electric motor (Fig. 1). The fuel dilution was less than half per cent. With 'leaded fuel', however, the permanent loss of viscosity was appreciably larger and increased with the lead content of the fuel. Yet, the increase in the temperature coefficient of the viscosity of the oil did not exceed in these cases the increase obtained when the engine had been driven by electric motor (Fig. 2). The temperature coefficient of the viscosity is here:

$$\alpha = \frac{\mathrm{d}\ (\log\ \log\ (\nu + 0{\cdot}6))}{\mathrm{d}\ (\log\ T)}$$

where $\nu$ is the kinematic viscosity in cS. and $T$ the absolute temperature. This could indicate that a greater number of large molecules was broken down in the presence of lead and that also the reactions

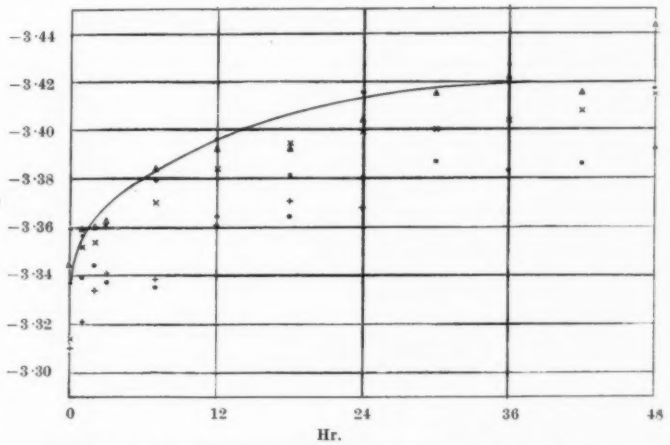


Fig. 2. Viscosity–temperature coefficient of used oil. ●, Engine 'motored'; ○, lead-free fuel; +, fuel containing 0·21 ml. tetraethyl lead/Imp. gallon; ×, fuel containing 1·85 ml. tetraethyl lead/Imp. gallon; △, fuel containing 3·57 ml. tetraethyl lead/Imp. gallon

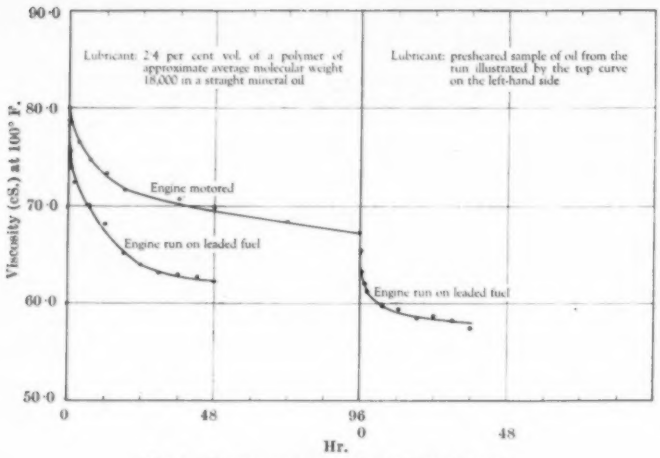


Fig. 3. Permanent loss of viscosity of lubricants

of the free radicals with oxygen molecules were promoted by the presence of lead. In fact, the sludge and acidity of the used oil showed a noticeable increase with the lead content of the fuel on which the engine had been run (Table 1).

Table 1

Sludge and acidity of used oil containing 1·2 per cent volume of a polymer of approximately average molecular weight of 18,000 in a straight mineral oil

| Lead content | Duration of run (hr.) | Naphtha insolubles (per cent) | Acidity (mgm. pot. hydrox./gm.) |
|---|---|---|---|
| 0 | 48 | 0·045 | 0·05 |
| 0·21 ml.* | 48 | 0·13 | 0·1 |
| 1·85 ml. | 48 | 0·16 | 0·18 |
| 3·57 ml. | 48 | 0·19 | 0·29 |
| Comparison of data from 'motoring' the engine | 48 | 0·06 | 0·05 |

* Lead content as ml. tetraethyl lead per Imperial gallon.

Table 2

Permanent loss of viscosity during motoring of an internal combustion engine

| Type of polymer | Approximate average molecular weight of polymer | Permanent loss of viscosity (per cent) |
|---|---|---|
| Polybutene | 900 | 0 |
| Poly*iso*butene | 18,000 | ~ 8 (~ 15 on doubling the concentration of the polymer) |
| Poly*iso*butene | 80,000 | ~ 43 |

Table 3

Comparison of the results obtained with an oil containing 2·4 per cent volume of a polymer of approximately average molecular weight 18,000 in a straight mineral oil before and after it had been sheared. In both cases the engine had been run on the same 'leaded fuel'

| Lubricant | Initial viscosity (cS.) at: 100° F. | 210° F. | Initial viscosity temperature coefficient | Final viscosity (cS.) at: 100° F. | 210° F. | Final viscosity temperature coefficient |
|---|---|---|---|---|---|---|
| New oil | 78·7 | 11·95 | −3·060 | 62·9 | 10·02 | −3·151 |
| Used oil from 'motoring' the engine for 96 hr. | 65·3 | 9·89 | −3·229 | 57·4 | 9·12 | −3·101 |

The dependence of the mechanical degradation of the oil for a given shearing stress upon the size and concentration of large molecules was confirmed when the engine was driven by an electric motor (Table 2).

Proof was also obtained that an oil the large molecules of which had previously been degraded by driving the engine for a prolonged period (96 hr.) with an electric motor suffered afterwards very little permanent loss of viscosity while running the engine on a 'leaded fuel' (Fig. 3). Its viscosity-temperature coefficient (Table 3), instead of further increasing, decreased noticeably, while the naphtha insolubles and the acid number increased significantly, from 0·12 to 0·24 per cent and from 0·18 to 0·32 mgm. potassium hydroxide/gm., respectively.

In all these experiments, the engine was run at a speed at which it was estimated that the mean rate of sheer in the main bearings was of the order of $10^7$ sec.$^{-1}$.

Robert Schnurmann
Ivor S. Stringer

Esso Research Ltd.,
Abingdon, Berks.

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## Growth of Sulphide Films on Steel

Sulphur and its compounds are widely used as 'extreme pressure' additives in gear oils. They can prevent scuffing of the gears by the formation of protective sulphide films. The kinetics of the formation of such films at high temperatures is of technological interest, and a study of this problem was made in this Laboratory, using radioactive tracers and electron microscopy.

In order to simulate the brief temperature flashes, which occur when gear teeth mesh, rectangular pulses of electric current were passed through a metal wire, a method of heating previously used in the Thornton Research Centre. The pulses lasted for 4 m.sec. and were followed by periods of 40 m.sec. during which no current was passed. The wire was kept immersed in liquid paraffin, containing sulphur or sulphur compounds, labelled with the isotope sulphur-35. After passing a number of pulses, the radioactivity acquired by the wire was measured. The film thickness was calculated by using a suitable calibration procedure. The wires were 2·5 cm. long and 0·025 cm. diameter and were of mild steel, stainless steel, 'Armco' iron, copper or brass. Pulse temperatures over the range 100–700° C. were used in the experiments. Only the results obtained with mild steel will be discussed here.

It was found that the results followed the generalized parabolic law of film growth[1]:

$$d^2 + k_1 d = k_2 t \qquad (1)$$

where $d$ is the film thickness and $t$ the time of reaction; $k_1$ and $k_2$ are constants. For the present experiments it was permissible to substitute $N$, the number of pulses for $t$, and the applicability of equation (1) was tested by plotting $d$ versus $N/d$. Straight lines intersecting the $d$-axis showed that the law was applicable,

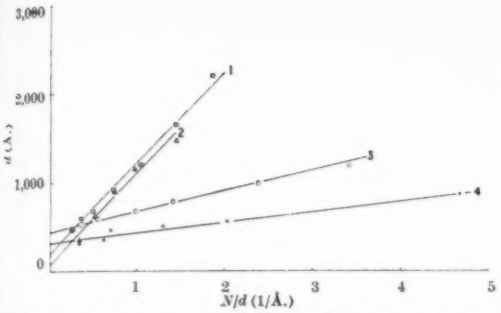


Fig. 1. $d$, film thickness; $N$, no. of pulses. Mild steel, pulse temperature. 400° C. 1, 2, dibenzyl sulphide; 3, 4, elemental sulphur

and this is illustrated in Fig. 1. Curves 1 and 2 relate to solutions of dibenzyl disulphide, containing the equivalent of 0·5 and 0·25 per cent by weight of sulphur. Curves 3 and 4 refer to elemental sulphur in similar concentrations. Mild steel wires and pulse temperatures of 400° C. were used in these experiments. The results can be interpreted as follows: during the first few pulses a fast reaction led to the build-up of relatively thick sulphide films (reaction-rate constant $k_1$). Soon, however, the reaction-rate slowed down and was then governed by a diffusion mechanism, characterized by the constant $k_2$. The coefficient of diffusion, $D$, and the energy of activation, $\Delta E_D$, could be calculated from the experimental results. For elemental sulphur solutions and mild steel at 400° C., $D$ was about $5 \times 10^{-12}$ cm.²/sec. and $\Delta E_D$ about 20 kcal./mole.

It seemed important to establish whether, during the initial stages, a uniform film developed over the surface or localized growth occurred. This was investigated using an *E.M.*6 Metropolitan-Vickers electron microscope. Two techniques were employed. Extraction replicas were prepared by evaporating carbon on to the surface of the wire. The film was removed by etching the wire for a short time in 10 per cent nital, then transferring it to distilled water where the film became detached from the wire and was collected in the usual way. Fig. 2*a* shows a micrograph obtained by this method. Apart from relatively thick regions, a large number of whiskers can be seen. Further information about the structure of the film was obtained by examining sulphided wires directly in the electron microscope. Fig. 2*b* shows the shadow cast by the edge of a wire. Large-scale growth of sulphide had occurred in a few places (marked *A*). The uppermost surface of these areas of growth shows the roots of the whiskers (marked *B*). The primary film of sulphide formed on the surface is shown not to be uniform and to consist of localized growth terminating in fine whiskers.

Fig. 2. (× *c.* 26,700).

A fuller account of the work will be published elsewhere.

R. B. Campbell
L. Grunberg
H. M. Scott

Lubrication and Wear Division,
National Engineering Laboratory,
Thorntonhall,
Glasgow.

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## METALLURGY

### An Iron–Silicon Carbide

Many microconstituents alleged to be iron carbides containing silicon have been reported[1–3]. Marles[4] carried out a systematic microscopic examination of rapidly cooled irons containing up to 7 per cent silicon and 1·7–3·6 per cent carbon. A non-heat-tinting constituent described as a 'silico-carbide' was found associated with cementite. Hurst and Riley[5] found a micro-constituent containing carbon and silicon in rapidly cooled iron–silicon–carbon alloys containing 10–15 per cent silicon. More recently, Owen and Street[6] identified another micro-constituent, believed to be a carbide containing silicon, in slowly cooled high-purity alloys containing 5–8 per cent silicon and small amounts of carbon. This carbide was designated the *X*-constituent.

Previous X-ray work[6] on these carbides failed to detect any similarities in their crystal structure, and the exact structure of each carbide was not ascertained. We have completed a detailed examination of the Marles carbide, the Hurst and Riley carbide and the *X*-constituent in solid and in extracted specimens. Using lithium fluoride monochromated radiation with a Debye–Scherrer technique, we find that all three carbides have the same crystal structure. The unit cell is hexagonal with $a = 11{\cdot}7$ Å., $c = 10{\cdot}8$ Å. and $c/a = 0{\cdot}925$, and no variation of these parameters with composition was detected.

We have attempted to determine the chemical composition of the hexagonal carbide by lineal analysis of selected micro-structures. In addition the carbide has been extracted and chemically analysed by the British Cast Iron Research Association. The general results suggest that the carbide contains approximately 9 per cent silicon and 6·5 per cent carbon, giving a formula for the carbide of $Fe_{10}Si_2C_3$. However, there is considerable scatter in the results, and this formula cannot be taken to be firmly established.

Harry[7] and Leslie *et al.*[8,9] have reported carbides other than cementite in low-carbon silicon steels,

containing up to 0·10 per cent carbon and between 3 and 4 per cent silicon. The reported X-ray diffraction and electron diffraction patterns cannot be reconciled with the pattern obtained from the hexagonal carbide reported here.

This work formed part of a larger research which will be described in the *Journal of the Iron and Steel Institute*. The work was supported by the Andrew Carnegie Research Fund.

J. G. Humphreys
Walter S. Owen

Department of Metallurgy,
University of Liverpool.

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## CHEMISTRY

### Ionic-Covalent Bonding in Crystals

In discussions in the literature regarding bonding in inorganic semiconducting (or insulating) crystals there seems to be some confusion between bond ionicity on one hand and charge separation effects due to electronegativity differences on the other. This appears to have arisen from the common assumption that the ionic component of bonding in solids is determined solely by the electronegativity difference ($\Delta x$) between the constituent atoms (that is, that a compound with, say, $\Delta x = 1{\cdot}0$ must be more ionic than one with $\Delta x = 0{\cdot}5$). Without some qualification, this assumption can be very misleading.

Some elementary arguments regarding electronegativity will serve as a useful introduction. This parameter, defined following Mulliken as the mean of the first ionization constant and the electron affinity of an isolated atom, can be considered a measure of relative electron attracting power. It is not, however, a constant since it depends on the orbital employed (for $s - p$ hybrids it has been shown to decrease as the orbital becomes more strongly directed[1]), on the degree of hybridization, and also on the effective ionic charge (increasing with positive charge, decreasing with negative[2]). Most existing scales of electronegativity, incidentally, take little account of these effects.

When atoms with different electronegativities combine, the more electronegative element tends to become negatively charged, which lowers its electronegativity, while the opposite is true of the less-electronegative element; at equilibrium both atoms have the same effective electronegativity[3]. Conversely, if before combination there is no electronegativity difference, no charge transfer will occur. These arguments apply equally to diatomic molecules and to the quasi-infinite molecules in ionic-covalent crystals.

It will be assumed in the following discussion, as a matter of definition, that by a covalent bond is understood one in which the bonding electrons are equally shared between the bonded atoms, and by an ionic bond one in which they are associated solely with the electronegative constituent[1,4]. It will also be assumed that continuous linear variation in bonding electron distribution is possible between the two extreme forms, and that effects associated with overlap can be neglected.

Applying the previous arguments to a typical ionic-covalent crystal, zinc sulphide, it follows that no charge separation would take place if zinc and sulphur, as free atoms, had the same electronegativities. In this case each atom in the lattice would have its normal complement of valence electrons associated with it (zinc two, sulphur six).

Such an electron distribution gives the 'neutral' formulation $Zn^0S^0$, which is to be compared with the extreme covalent formulation $Zn^{2-}S^{2+}$ (equal sharing of the electrons, see, for example, Pauling[4]), and ionic formulation $Zn^{2+}S^{2-}$ (electrons associated solely with the electronegative constituent).

It will be noted that, lying exactly midway between these extremes, the 'neutral' bonds of $Zn^0S^0$ would be formally 50 per cent ionic (or 50 per cent covalent), that is, with equal contributions from covalent and ionic forms.

Neutral bonds of this type, however, would only be formed in the absence of any difference in electronegativities. Sulphur has in fact a considerably higher electronegativity than zinc, so that in crystalline zinc sulphide charge separation will take place with respect to the neutral bond state, the sulphur atoms becoming negatively charged and the bonds becoming more than 50 per cent ionic.

This simple approach (which appears first to have been pointed out some years ago[5]) is capable of general application to ionic-covalent solids (and also to dative bonds in simpler molecules), since in all cases the neutral bond state is the natural zero point for charge separation arising from electronegativity differences. If only one type of bond is present in the lattice the neutral bond state can be deduced by inspection. Thus for all $A^{II}X^{VI}$ compounds (of which zinc sulphate is an example) it occurs at 50 per cent ionic bonding, for $A^{I}X^{VII}$ compounds such as sodium chloride (covalent form $A^{I0}X^{VII0}$, ionic form $A^{I+}X^{VII-}$) at 75 per cent, and for $A^{III}X^{V}$ compounds such as aluminium nitride (covalent form $A^{III0}X^{V0}$, ionic form $A^{III3+}X^{V3-}$) at 25 per cent. For more complex compounds in which more than one type of bond is present each type can be assigned its specific neutral-bond ionicity independently. The actual bonds in compounds will of course depart from the neutral form(s) to an extent dependent on the appropriate electronegativity difference(s).

This raises the problem of what scale of electronegativity is applicable to solids. It is not proposed to discuss this matter in any detail, but the following observations appear pertinent.

In principle there is a separate electronegativity scale for every type of bonding orbital, equally applicable to atoms in solids or in simpler molecules. The relative order of the elements should show little variation from scale to scale, but, as mentioned above, absolute electronegativity values can change with directivity or hybridization of bonding orbital. It would seem, however, that even between extreme cases this change is unlikely to exceed 0·5 unit, so that Pauling-type scales are probably not grossly misleading (particularly for large electronegativity differences) if one wishes to obtain some idea of the sign and relative extent of charge separation. (It should be noted, however, that there are in any event

errors arising in determining Pauling type electronegativities which are probably of the order of $\pm 0{\cdot}1$ unit or more.) The Sanderson scale[6,7], for tetrahedral co-ordination, also appears of interest since it is apparently derived for the case of a single bonding orbital.

Finally, even for simple molecules there appears to be no reliable relationship between charge separation and electronegativity difference, and little attempt has been made to establish one for solids. In this connexion the alkali halides, with an electronegativity difference of $\sim 2$ units, must presumably show separation of, effectively, a whole electronic charge. Perhaps values for smaller electronegativity differences may eventually be deduced from restrahlen frequency measurements, but, as pointed out by Matossi[8] and others, this approach is not promising. For the present one can only assume a monotonic increase of charge separation with electronegativity difference.

Some examples to illustrate these points follow:

Consider the *iso*electronic pair sodium fluoride ($A^{I}X^{VII}$ group) and magnesium oxide ($A^{II}X^{VI}$ group). Neutral-bonded sodium fluoride would be 75 per cent ionic (see above); the large difference in the electronegativities of sodium and fluorine brings about the transfer of presumably about a whole unit of charge to make the real sodium chloride lattice almost 100 per cent ionic. In contrast neutral-bonded magnesium oxide is 50 per cent ionic, and, with a much smaller electronegativity difference than for Na—F, the real magnesium oxide lattice is unlikely to be even 75 per cent ionic (the appreciable covalency of its bonds gives magnesium oxide very different properties from sodium fluoride, for example hardness).

With regard to semiconducting compounds the present approach indicates that III–V zinc blende compounds are all more than 25 per cent ionic while the related II–VI compounds (like zinc sulphide) must be more than 50 per cent ionic. This is in agreement with microcleavage data[9] and, for the case of gallium arsenide, with estimates based on an analysis of spin-orbit splitting in infra-red transmission spectra[10].

Pyrites ($FeS_2$) is a simple example of more complex compounds in which more than one kind of bond may be present. The S—S bonds in pyrites can be assumed covalent, leaving 12 electrons for 6 Fe—S bonds[11]. Only two of these 'belong' to Fe, so that the neutral form of the Fe—S bond is 2/6 (that is, 33·3 per cent) covalent. The actual difference in the electronegativities of Fe and S will further reduce this covalent contribution in the real lattice, but will have no comparable effect on the S—S bonds.

Generally compounds tend to be more ionic than the neutral-bonded form since, as would be expected, 'anionic' elements usually have higher electronegativities than 'cationic' elements. (It should be noted that HgTe may be an exception since the appropriate electronegativity difference is only 0·2 unit, which is within the limit of accuracy of available electronegativity scales. Similar arguments may also apply to $As_2Te_3$. Electronegativity values are taken from Haïssinsky[12].)

From many points of view it would appear useful to differentiate two ionic components of bonding in solids: structural ionicity associated with a neutral-bond structure, and charge separation ionicity arising from electronegativity differences. It is the second of these which is responsible for optical scattering of charge carriers in semiconductors, and, to some extent at least, for the difference between high- and low-frequency dielectric constants in 'ionic' crystals (in connexion with the latter effect, the simple approach here outlined should enable the sign of the charge separation to be deduced, a matter of argument in the past). Both components must be taken into account when considering bond-strength and related properties.

C. H. L. Goodman

Research Laboratories,
The General Electric Co., Ltd.,
Wembley, England.

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## Optically Active Forms of a Derivative of Tetra-substituted Sulphur

The concept of the configuration of unevenly tri-substituted sulphur derivatives as an incipient tetrahedron is borne out by both physical studies and resolutions; but the evidence for the tetrahedral structure of tetra-substituted sulphur rests solely on physical investigations.

The simplest member of a new class of sulphur compound, dimethyl sulphoximine, was first prepared by permanganate oxidation of *SS*-dimethyl-*N*-toluene-*p*-sulphonylsulphidimine to *SS*-dimethyl-*N*-toluene-*p*-sulphonylsulphoximine, followed by acid hydrolysis[1]. As part of a study of an optically active chloramine-*T*, one stereoisomer of sodium-4-(3-menthyl)-benzenesulphonchloramide, it seemed of interest to attempt its conversion to the sulphoximide of a mixed sulphide in order to investigate possible isomerism of the *dC.dS.* and *dC.lS.* type, suggested by the following facts. The sulphur–nitrogen linkage, in the sulphidimine, and the sulphoxide bond are stereochemically analogous[2,3], and oxidation of the latter gives sulphones which are known to have an approximately tetrahedral structure[4].

Model experiments confirmed the previous observations of the instability of sulphidimines of mixed sulphides, bearing more complex substituents than dimethyl, under oxidizing conditions[3,5]. Chloramine-*T* and *p*-nitrophenylmethylsulphide gave the normal sulphidimine, m.p. 161°; identical material resulting from the condensation of *p*-toluenesulphonamide with *p*-nitrophenylmethyl sulphoxide, in presence of phosphorus pentoxide[6]. Treatment of this sulphidimine with permanganate under various conditions only gave starting material or *p*-toluenesulphonamide.

The oxidation product was eventually obtained by condensing, in dry pyridine, toluene-*p*-sulphonylchloride with the basic *p*-nitrophenylmethylsulphoximine[7] (prepared by the more general route of treating the sulphoxide with hydrazoic acid), giving the required sulphoximide as a neutral stable compound, m.p. 203–204°. The infra-red spectra showed nitro-

adsorption at 6·6 and 7·4$\mu$ and strong bands at 7·7, 8·1, 8·7 and 9·2$\mu$, which are also the principal bands in the spectra of the *SS*-dimethyl-*N*-toluene-*p*-sulphonylsulphoximine, and compared with the spectra of the *p*-nitrophenylmethylsulphidimine showed shifts in the position of the major bands identical to those observed between the spectra of the dimethyl analogues.

Condensation of the optically active 4-(3-menthyl)-benzenesulphonylchloride, m.p. 13°, $[\alpha]_{5461}^{24} + 64·6°$, obtained by suitable treatment of an optically pure strychnine 4-(3′-menthyl)-benzenesulphonate with *p*-nitrophenylmethylsulphoximine gave *S*-*p*-nitrophenyl-*S*-methyl-*N*-[4-(3-menthyl)-benzenesulphonyl]-sulphoximine as a mixture of diastereoisomerides, m.p. 133–136°, $[\alpha]_{5461}^{24} + 34·0°$, which showed large changes of rotatory power on recrystallization. The less-soluble form, thick hexagonal plates from methanol–chloroform, had m.p. 150°, $[\alpha]_{5461}^{21} - 155°$, $[\alpha]_{D}^{21} - 124°$. (Found: C, 57·7; H, 6·2; N, 6·0; S, 13·5. $C_{22}H_{30}O_5N_2S_2$ requires C, 57·7; H, 6·3; N, 5·9; S, 13·4 per cent). Systematic fractionation of the mother-liquor has given an extreme crop, m.p. 138–140°, $[\alpha]_{5461}^{19} + 171·9°$, $[\alpha]_{D}^{19} + 138°$. (Found: C, 57·6; H, 6·3; N, 6·1; S, 13·1 per cent.) The infra-red spectra of the diastereoisomers were almost identical and showed the characteristic sulphoximide adsorption.

These results give an indication of asymmetry at the tetrasubstituted sulphur atom and therefore it seems that, like the sulphones and sulphuryl halides[4], this class of sulphur compound has a tetrahedral structure.

I thank the Council of Bedford College for a post-graduate studentship, Dr. D. G. H. Daniels, and the Research Director of the Research Association of the British Flour-Millers for specimens of the dimethyl sulphidimine and sulphoximide for infra-red comparison, and Mr. J. F. Robinson for technical assistance.

M. Barash

Bedford College,
University of London.

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## A New Method for the Synthesis of Cyclic Ethers

Heterocyclic compounds having four members in the ring are difficult to prepare, and are generally less well known than the 3-, 5- and 6-membered rings of the same type. In the cyclic ethers, for example, oxetanes have been studied relatively little compared to oxiranes and tetrahydrofurans.

The oxetanes have considerable potential as chemical intermediates and solvents provided that they could be made economically. For example, oxetane polymers, particularly from 2,2-disubstituted oxetanes, have interesting properties. However, most of the known syntheses of oxitanes are tedious, expensive and give moderate yields, at best[1]. The only exceptions are the ring closures involving the trihalo derivatives of pentaerythritol[2] and the pyrolysis of hydroxy-cyclic carbonates[3].

Recently[4], a new, general synthesis of oxetanes has been reported. This synthesis is a direct, cheap process giving fair yields, and it has been further investigated to determine its scope and limitations.

The synthetic procedure is simple. A diol with the hydroxy groups separated by 3 carbons is dissolved in concentrated sulphuric acid. The mixture is dropped into a boiling solution of aqueous caustic. The product oxetane distils over as it is formed. The distillate separates into two layers, as a general rule. The ether is salted out of the aqueous phase, dried over calcium hydride, and distilled. The procedure is also useful for the preparation of other cyclic ethers, such as tetrahydrofurans and tetrahydropyrans.

The various oxetanes which have been made by this procedure are listed in Table 1. In all runs, 55·5 ml. of 98 per cent sulphuric acid was used to dissolve the diol.

Table 1. Cyclic Ethers

| Diol (gm.) | Caustic (gm. and per cent) | Product (gm.) | Boiling point (° C.) |
|---|---|---|---|
| Neopentyl glycol (104) | 240 (55) | 2,2-dimethyl oxetane (24·5) | 79·2–80·3 |
| 2-Butyl, 2-ethyl 1,3-propanediol (142) | 240 (55) | 2-butyl, 2-ethyl oxetane (44·4) | 181·2–183·9 |
| 1,3-Butanediol (90) | 240 (64) | 1-methyl oxetane (about 15) | 58–61 |
| 2 Ethyl-1,3 hexanediol (146) | 240 (55) | 1-*n*-propyl, 2-ethyl oxetane (24·3) | 152·5–155·5 |
| 2,2-Diethyl 1,3-propanediol (132) | 240 (55) | 2,2-diethyl oxetane (38·3) | 139–141 |
| 1,4 Butanediol (90) | 240 (55) | tetrahydrofuran (47·1) | |
| 1,5 Pentanediol (104) | 240 (55) | tetrahydropyran (35·4) | |
| 2,5-Dimethyl, 2,5-hexanediol (146) | 240 (55) | 2,2,5,5-tetramethyl tetrahydrofuran (87·3) | 110–112 |

In an attempt to obtain an optimum yield of 2,2-dimethyl oxetane from this synthesis, a factorial experiment was run, in which the factors were the following:

Acid, strength: 98 per cent, oleum.
Acid, quantity: equimolar, 50 per cent excess with respect to glycol.
Base, strength: 40 per cent, 70 per cent.
Base, quantity: stoichiometric, 50 per cent excess with respect to acid.

The results of the experiment, together with replications, are given in Table 2. The values are grams of product obtained from 208 gm. neopentyl glycol as starting material.

Table 2. Factorial Design used to determine Significance of Variables

| Sodium hydroxide | | Sulphuric acid | | | |
|---|---|---|---|---|---|
| | | $H_2SO_4$ | | $H_2SO_4$-Oleum | |
| | | Stoichiometric | 50 per cent excess acid | Stoichiometric | 50 per cent excess acid |
| Initial 70 wt. per cent conc. | 50 per cent excess caustic | 49·2 | 37·1 | 48·1 | 36·4 |
| | Stoichiometric | 46·0<br>16·2 | 31·3<br>19·9 | 13·1 | 14·6 |
| Initial 40 wt. per cent conc. | 50 per cent excess caustic | 7·8 | 11·5 | 5·6 | 13·7<br>21·5 |
| | Stoichiometric | 7·0 | 2·0<br>2·3 | 2·0 | 3·9 |

An analysis of variance of the factorial experiment shows that only the amount of caustic, the concentration of caustic, and their interaction have significant effects on the yield of oxetane.

Because of the nature of the process, it is felt that the instantaneous concentration of caustic is the variable actually affecting the yield of oxetane. The amount of excess base only affects the range of concentrations during a run. In another experiment, a number of runs were made at varying concentra-

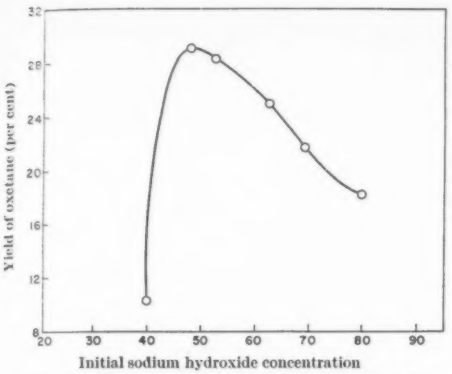


Fig. 1. Effect of sodium hydroxide concentration upon the yield of 3,3-dimethyloxetane

tions of caustic. In each case, 200 per cent excess caustic was used so that the concentration of base remained fairly constant during the run. The results are given in Fig. 1. It is seen that the maximum yield of oxetane occurs at an initial concentration of base of about 50 per cent.

Since the yield of oxetane is relatively low, and since only small amounts of non-condensable by-products were noted, the composition of the residual solution was briefly investigated. In one experiment, using 104 gm. neopentyl glycol, 55·5 ml. sulphuric acid, and 480 gm. of 50 per cent sodium hydroxide, 21·2 gm. of dimethyl oxetane was produced. The residual solution was neutralized with sulphuric acid and extracted with ether. A total of 38 gm. of material was recovered from the extracts. This material appeared to be substantially unchanged neopentyl glycol.

In another experiment, using 208 gm. neopentyl glycol, 111 ml. sulphuric acid and 440 gm. of 54·5 per cent caustic, 53 gm. of dimethyl oxetane was produced, and 73 gm. of crude glycol was recovered by a chloroform extraction of the diluted and filtered residue. The recovered glycol was distilled, and 50 gm. of the material came over between 200° and 207° C. A few grams of residue remained when decomposition began. The results of the two experiments are seen to be quite similar. In the latter experiment, 135 gm. of glycol was consumed, a conversion of 65 per cent; the yield of product is 53 gm., or 47 per cent, based on the amount of glycol actually used up.

It was noted that additional material could be recovered by a chloroform extraction of the residual solution, after the solution had been neutralized with sulphuric acid. This material was recovered as a viscous liquid, which slowly crystallized, and was clearly not neopentyl glycol. Because of the limited solubility of this material in chloroform, it was not quantitatively recovered.

About 30 gm. of crystalline material, of moderate solubility in water, insoluble in chloroform and acetone, and having an infra-red spectrum consistent with an ionic sulphonate, was isolated from the run in the factorial experiment made with 50 per cent excess oleum, and 50 per cent excess of 70 per cent caustic. This material was not further identified. Presumably, the formation of this, and similar materials, accounts for the greater part of converted glycol not appearing as oxetane. As already noted, very little volatile by-product is formed. Further work is in progress to identify these side-products and to obtain a complete material balance, as well as to improve the yields of cyclic ethers.

L. F. Schmoyer*
L. C. Case

Department of Chemical Engineering,
Purdue University,
Lafayette, Indiana.

* Present address: Atlas Powder Company, Wilmington, Delaware.

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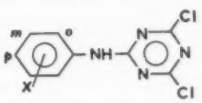
## An *Ortho* Effect influencing Fungitoxicity, Electronic Absorption Spectra, and Chemical Reactivity with Mercaptalbumin

The derivative of 2,4-dichloro-6-anilino-*s*-triazine containing a chlorine atom in the benzene ring *ortho* to the imino group is less toxic to conidia of *Neurospora sitophila* (Mont.) Shear and Dodge than the corresponding *meta* or *para* isomer[1].



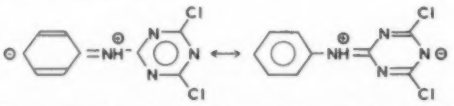

The principal effect of *ortho* substitution is to reduce the amount of toxicant taken up by the fungus spores from the ambient solution, the *ED*50 values of the three compounds being approximately the same on a spore-weight basis. More recent results obtained on conidia of *N. sitophila* reveal that the same order of uptake is followed when the substituent group is methyl or ethoxy (Table 1), and that substitution of the imino hydrogen on the bridge connecting the aromatic and heterocyclic rings with a methyl group also reduces accumulation of the chemical by the spores. The effect is greatest when both an *ortho* hydrogen and the imino hydrogen are replaced by methyl groups. In this case only 15 per cent of the applied dose is taken up compared to 77 per cent for the *p*-methyl derivative. The same pattern is also found for the uptake of these compounds by conidia of *Myrothecium verrucaria* (Alb. and Schw.) Ditm. ex Fr., *Glomerella cingulata* (St.) Sp. and von S., and *Aspergillus niger* van Tiegh.

These observations suggest that maximum uptake of the triazine is obtained when the aromatic and heterocyclic rings can assume a coplanar configuration, since bulky groups in an *ortho* position of the benzene ring or on the bridge nitrogen would interfere with free rotation through van der Waals repulsion

Table 1. Influence of Isomerism on the Uptake of *s*-Triazine Derivatives by Conidia of *N. sitophila* at Applied Doses of 6 Micromols per Gram of Spores

| Substituent(s) | Percentage of isomer taken up: *Ortho* | *Meta* | *Para* |
|---|---|---|---|
| Cl | 81 | 86 | 87 |
| $C_2H_5O$ | 55 | 78 | 74 |
| $CH_3$ | 53 | 75 | 77 |
| $N—CH_3$, $CH_3$ | 15 | 40 | 48 |

forces and steric hindrance. An alternative hypothesis is that *ortho* substituents might decrease binding to metabolic substrates by interfering with the formation of hydrogen bonds between the imino group and electron- or proton-donating groups at receptor sites within the spores. In either case it should be possible to demonstrate crowding at the imino bridge through changes in electronic absorption spectra, since substituents that reduce degree of coplanarity would concomitantly reduce interannular resonance. This would result in a shift of absorption maxima to shorter wave-lengths and lower molar extinction coefficients, since absorption of radiation by these compounds arises predominantly from $\pi - \pi^*$ transitions. In particular, the weights of ionic valence-bond structures of the following type in predicting electron density distribution in the resonance hybrid would be reduced:



On comparing the electronic spectra of *s*-triazines substituted in various positions with methyl groups, it can be seen that *ortho* substitution produces a hypsochromic shift of 32 mμ coupled with lowering of intensity when the solvent is acetonitrile (Table 2). Substitution on the imino group causes a blue shift of 25 mμ, but the molar extinction is not changed appreciably. In *cyclo*hexane solution, the blue shift arising from *ortho* substitution is only 3 mμ, but the molar extinction coefficient is reduced by 20 per cent. The *N*-substituted isomer exhibits a pronounced blue shift in both solvents. By contrast, small red shifts occur in both the polar and non-polar solvents when methyl groups are substituted for hydrogen in the *meta* or *para* positions. Similar results are obtained when chlorine atoms and ethoxy groups are substituted in the aromatic ring, indicating that bulky *ortho* substituents reduce conjugation. This is highly correlated with the amounts of these compounds that are taken up by fungus spores.

Table 2. Electronic Absorption Data on *s*-Triazine Derivatives in *cyclo*Hexane and Acetonitrile

| Substituent | Acetonitrile $\lambda_{max.}$ | Acetonitrile $\varepsilon$ | *Cyclo*hexane $\lambda_{max.}$ | *Cyclo*hexane $\varepsilon$ |
|---|---|---|---|---|
| None | 269 | 17,700 | 271 | 18,900 |
| *o*-Methyl | 237 | 14,200 | 268 | 13,300 |
| *m*-Methyl | 271 | 16,900 | 275 | 19,300 |
| *p*-Methyl | 273 | 17,500 | 276 | 19,900 |
| *N*-Methyl | 244 | 17,200 | 245 | 16,100 |

Concurrent studies on the kinetics of the nucleophilic substitution reactions that take place between the *s*-triazines and bovine mercaptalbumin[2] indicate that an *ortho* effect occurs here as well, the *o*-methyl isomer being only about 10 per cent as reactive with the protein as the *meta* and *para* isomers. It is unlikely that this results from a higher energy of activation in the reaction of the *ortho* isomer with mercaptalbumin, since the second-order velocity coefficients for the reactions of the three isomers with glycine are about the same. Steric effects are unlikely to be involved to a major extent in this latter case, because of the small size of the glycine molecule. Therefore the net electron-releasing power of the methyl group, arising from induction and hyperconjugation, has the same order of magnitude at all three positions in the aromatic ring, leaving steric hindrance as the only way to account for the low reactivity of the *ortho* isomer with the protein.

At present it is not clear whether the *ortho* effects demonstrated in the interactions of the *s*-triazines with fungus spores and the animal protein arise from a reduction in degree of coplanarity of the rings, or steric hindrance of hydrogen bonding at the imino group, or both. However, the possibility that *s*-triazines of this type can form hydrogen bonds with metabolic substrates is supported by the finding that the *o*-methyl isomer is significantly less reactive with tyrosine than the *meta* and *para* compounds, perhaps because hydrogen bonding of the imino groups of the latter molecules with the phenolic hydroxyl group of the tyrosine speeds up the reactions by a Michaelis–Menton type mechanism.

H. P. Burchfield
Eleanor E. Storrs

Boyce Thompson Institute,
Yonkers, New York.
April 6.

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## RADIOCHEMISTRY

### Non-destructive Analysis of Selenium by Neutron Activation followed by Gamma-Ray Spectrometry

Detection of selenium by neutron irradiation and gamma scintillation spectrometry has been attempted. It is known that, when natural selenium is irradiated with neutrons, selenium-75 (121 days), selenium-77 *m* (17·5 sec.) and selenium-81 (18·2 min.) are formed with comparatively large cross-sections. When any one of them is used to detect selenium, irradiation must be continued for a period comparable to the half-life. Therefore, it was thought that the use of selenium-77 *m* offered a method for the rapid detection of selenium; this method has not been described in the literature examined.

In the present work, the sample (0·1–0·5 gm.) was irradiated at a neutron flux of $3 \times 10^{11}$ neutrons per sq. cm. per sec. for 20 sec., cooled for 15 sec., counted for 20 sec., again cooled for 20 sec. and counted for 20 sec. The counting apparatus used was a 256-channel pulse-height analyser with a 2-in. thick, well-type sodium iodide crystal. A typical spectrogram thus obtained is shown in Fig. 1, from which it is seen that: (1) there is only one photopeak;

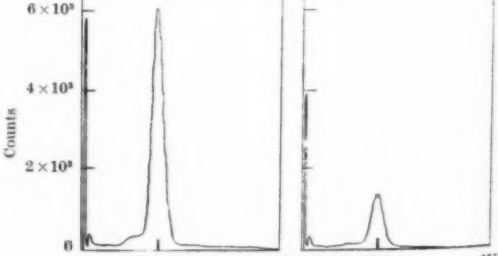


Fig. 1. Typical spectrogram. Sample, 0·109 gm. of sulphur powder. Energy scale ≃ 3·3 keV./pulse-height unit

Table 1. RADIONUCLIDES FORMED, THE GAMMA-RAY ENERGY OF WHICH IS ABOUT 0·16 MEV. (ARRANGED BY INCREASING HALF-LIFE)

| Nuclide formed | Half-life | Nuclide formed | Half-life |
|---|---|---|---|
| $^{187m}$W | 5·5 sec. | $^{233}$Th | 22 min. |
| $^{77m}$Se | 17·5 sec. | $^{131}$Te | 25 min. |
| $^{179m}$Hf | 19 sec. | $^{123}$Sn | 40 min. |
| $^{77m}$Ge | 54 sec. | $^{111m}$Cd | 49 min. |
| $^{165m}$Dy | 1·25 min. | $^{139}$Ba | 84 min. |
| $^{187}$W | 1·62 min. | $^{177}$Yb | 1·9 hr. |
| $^{161}$Gd | 3·6 min. | $^{85m}$Kr | 4·4 hr. |
| $^{182m}$Ta | 16·5 min. | $^{186}$Re | 17 hr. |
| $^{76}$Ga | 21 min. | | |

(2) the corresponding gamma-ray energy is in the range 0·15$_5$–0·17 MeV.; (3) the half-life of the photopeak is about 17·5 sec.

Radionuclides formed by neutron irradiation from naturally occurring nuclides, and with gamma-ray energy of about 0·16 MeV., are shown in Table 1.

Of these radionuclides, those which show similar shapes of the gamma-spectra of Fig. 1 are selenium-77 *m*, tantalum-182 *m*, tin-123 and barium-139. Their half-lives are 17·5 sec., 16·5 min., 39·5 min. and 84 min., respectively.

By the comparison of observed half-life with the values given above, selenium can be identified, and it is confirmed that the observed photopeak is due to selenium.

Table 2. RESULTS OF ANALYSES

| Sample | Selenium found (p.p.m.) |
|---|---|
| *A* (Sulphur) | 68 |
| *B* (Sulphur ore) | 6·8 |
| *C* (Ammonium thiocyanate) | < 0·02 |
| *D* (Ammonium sulphate) | < 0·05 |

For the purpose of quantitative determination, the relation between photopeak height and the amount of selenium can be obtained by use of standards containing selenium in various amounts ranging from 0·1 to 100 μgm. (see Table 2).

I am indebted to Prof. Shozi Makishima for helpful suggestions, to Dr. Toshio Nakai for permission to use laboratory facilities, and to Dr. Seishi Yajima for help with the gamma-spectra.

MINORU OKADA

Government Chemical Industrial
Research Institute,
Shibuya-ku, Tokyo.
April 4.

## BIOCHEMISTRY

### Reactivity of the Disulphide Bond in Wool

THE permanent set which strained wool fibres acquire in steam or boiling water is due to breakdown of disulphide bonds; this promotes dissipation of stress, followed by linkage rebuilding, which fixes the relaxed structure in its deformed state. In such conditions oxidized fibres are more difficult to set than untreated, and it has been suggested that the formation of intermediate oxidation products, such as the sulphone, is sufficient to impair setting power[1]. This view has now been confirmed by taking advantage of the observation that sulphur dioxide can be reduced to sulphur by hypophosphorous acid[2].

Lincoln wool fibres were treated with a large excess of 2-vol. hydrogen peroxide for 3 hr. at *p*H 9·4 (borate buffer) and 50° C. After overnight washing in running water, the fibres were rinsed in several changes of distilled water, immersed in 0·05 *M* hypophosphorous acid at 50° C. for various times, and again washed to remove the acid. Fibres from each lot of wool were then stretched 40 per cent in cold distilled water, immersed in boiling distilled water for 1 hr., and finally released in boiling distilled water for 1 hr. Values for the set retained by the fibres are given in Table 1; corresponding values for unoxidized fibres, which had been treated with hypophosphorous acid under similar conditions, are also included. Each result is the average of the values obtained with at least three fibres.

Table 1

| Time of treatment with hypophosphorous acid (min.) | Percentage set of: | |
|---|---|---|
| | Untreated fibres | Oxidized fibres |
| 0 | 9·7 | −6·2 |
| 75 | — | 3·4 |
| 150 | — | 6·2 |
| 180 | 17·0 | — |
| 270 | — | 7·2 |
| 400 | — | 9·4 |
| 660 | 17·8 | 12·9 |
| 960 | 19·4 | 16·8 |

It seems clear that the ability of hypophosphorous acid to restore setting power to oxidized fibres is due to the reduction of intermediate oxidation products to disulphide cross-linkages, because, whereas the oxidized wool failed to blacken during boiling for 40 min. in lead acetate solution at *p*H 4·9 (ref. 3), the same wool, after treatment with hypophosphorous acid for 16 hr., blackened rather more than untreated wool. This result cannot be due to the reduction of cystine cross-linkages to cysteine side-chains, because wool fibres do not supercontract, and strained fibres fail to acquire a set, on being boiled for 30 min. in 0·05 *M* hypophosphorous acid.

The effect of hypophosphorous acid in increasing the setting power of untreated fibres must likewise be due to the reduction of the intermediate oxidation products formed by the action of light and air on the cystine linkages during the growth of the fibres. The presence of such oxidation products to variable degrees in normal fibres, and to different extents in different wools, depending on the conditions to which they were exposed during growth, is one cause of the erratic results obtained in setting fibres under conditions (steam or boiling water) which are far removed from the optimum. Their presence may provide a reason, also, for the failure to account for the whole of the sulphur in keratin in terms of cystine, cysteine, lanthionine and methionine.

As regards the chemical mechanism of setting, the differences of behaviour between untreated and oxidized fibres provide a striking proof that the

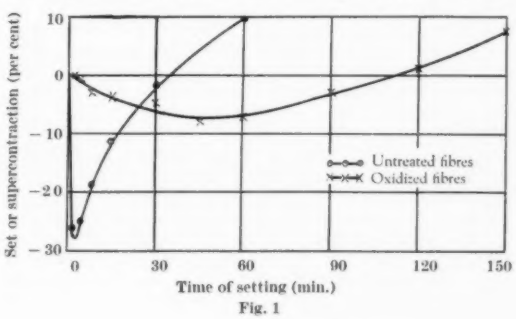


Fig. 1

breakdown of disulphide bonds is an essential first step. As before, the fibres were stretched 40 per cent, and after being immersed in boiling water for various times were released in boiling water for 1 hr. The amounts of set retained by untreated and oxidized fibres are shown in Fig. 1. As would be expected, in view of the greater stability of the intermediate oxidation products of cystine in boiling water[4], the oxidized fibres supercontract to a much smaller extent and are set much more slowly than the untreated fibres, which contain a high proportion of the more vulnerable cystine cross-linkages. In the light of these results, there can be little doubt that the failure of fibres treated with nitric acid to supercontract and set in boiling water[5] is due mainly to the oxidation of the cystine cross-linkages to more stable sulphones.

Finally, it should be mentioned that oxidized fibres can be set readily in boiling dilute solutions of sodium bisulphite, borax and sodium hypophosphite, in accordance with the fact that the intermediate oxidation products of cystine undergo fission readily in bisulphite and alkalis.

L. J. Wolfram
J. B. Speakman

Department of Textile Industries,
University, Leeds.
March 29.

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## Structures of Some Acetyl-serine Peptides from Acetyl-chymotrypsin

In 1958, two of us[1] obtained, by enzymic degradation of acetyl-chymotrypsin labelled with carbon-14[2], five peptides containing labelled acetyl-groups. Subsequent sequence studies[3] have shown that the largest of these peptides has the amino-acid sequence, Gly·Asp·Ser·Gly·Gly·Pro·Leu, identical with that already found[4], by degradation of the di-*iso*propyl-phosphoryl-enzyme, to surround the reactive serine; it may reasonably be concluded that the other four peptides contain the sequences Ser·Gly·Gly·Pro·Leu, Asp·Ser·Gly·Gly, Gly·Asp·Ser·Gly and Ser·Gly·Gly, respectively.

Since such studies cannot, on their own, show whether the acetyl-group is attached to an amino- or a hydroxyl-group in the serine residue, two others of us[5] synthesized the four peptides, (I)–(IV): (I) Ac·Ser(OH)·Gly·Gly·OH, (II) H·Ser(OAc)·Gly·Gly·OH, (III) Ac·Ser(O·Asp·H)·Gly·Gly·OH, (IV) H·Asp·Ser(OAc)·Gly·Gly·OH, with the object of comparing them with two of the smaller peptides obtained from acetyl-chymotrypsin. We now report the results of our joint work; the details of the synthetic work and of the sequence studies will be published elsewhere[3,5].

Owing to the low yields in which the smaller peptides are obtained by degradation of acetyl-chymotrypsin, direct comparison with the synthetic products was not possible. However, the behaviour of the two synthetic acetyl-tripeptides on electrophoresis (Table 1) clearly shows that, as is to be expected, only the N-acetyl-tripeptide (I) resembles the acetyl-tripeptide from acetyl-chymotrypsin in being negatively charged at *p*H 3·6 as well as at *p*H 6·5. Clearly the acetyl-tripeptide from acetyl-chymotrypsin is N-acetyl-seryl-glycyl-glycine (I), and not the O-acetyl-compound (II).

Table 1. Electrophoresis of Some Acetyl-serine Peptides

| Peptide | *p*H 3·6 | *p*H 6·5 |
|---|---|---|
| (I) | + 5·5 | + 17·8 |
| (II) | − 4·4 | + 5·2 |
| (III) | + 2·5 | + 15·0 |
| (IV) | + 2·4 | + 15·2 |
| Glucose control | 0·0 | + 3·5 |

Paper electrophoresis, in pyridine–acetate buffer, for 1 hr. at 2,000 V./40 cm. The figures are distances migrated, in mm., towards the anode (+) or cathode (−).

It will be seen, from Table 1, that the two acetyl-tetrapeptides (III) and (IV) are electrophoretically indistinguishable. They were equally indistinguishable by paper chromatography in a number of solvent systems, and recourse had, therefore, to be made to reaction with hydroxylamine as a method for distinguishing such isomeric pairs of acetyl-peptides; this reagent splits the O-acyl linkage, only O-acetyl peptides giving the readily detected acetohydroxamic acid.

The experimental procedure was as follows. A solution of hydroxylamine was prepared by mixing solutions of potassium hydroxide (560 mgm.) in methanol (5 ml.) and of hydroxylamine hydrochloride (347 mgm.) in methanol (5 ml.) and removing the precipitated potassium chloride by centrifugation. This reagent (10 μl.) was heated for 1 min. at 100° with 50 μl. of an aqueous solution containing the acetyl-peptide (0·1 μmole); after cooling, a few drops of methanol were added and the resulting solution acidified (*p*H 6). After paper chromatography, acetohydroxamic acid was detected by spraying with an alcoholic solution of ferric chloride (1 per cent) and hydrochloric acid (1 per cent), appearing as a red spot, $R_F = 0{\cdot}89$ (in *t*-BuOH : $H{\cdot}CO_2H$ : $H_2O$; 70 : 15 : 15), 0·45 (in *n*-butanol saturated with water); in the case of acetyl-peptides labelled with carbon-14, the chromatograms were also kept in contact with Kodak X-ray film, material containing carbon-14 being detected by darkening of the developed film. Appropriate markers were run in all cases.

Under these conditions, the synthetic O-acetyl-peptides, (II) and (IV), gave acetohydroxamic acid; the N-acetyl-tripeptide, (I), gave no hydroxamic acid, while the O-aspartyl-N-acetyl-peptide, (III), gave a hydroxamic acid, $R_F = 0{\cdot}26$ and 0·06 (in the two solvent systems), which was undoubtedly aspartyl-α-hydroxamic acid. Treated in the same way, the acetyl-pentapeptide ($R_F = 0{\cdot}94$ and 0·06) from acetyl-chymotrypsin gave no acetohydroxamic acid, the only radioactive material in the reaction product being the unchanged pentapeptide which is, therefore, an N-acetyl-compound; the acetyl-heptapeptide ($R_F = 0{\cdot}80$ and 0·01), however, gave acetohydroxamic acid as the sole radioactive reaction product; clearly, this peptide is an O-acetyl-compound.

Summarizing our results, it appears that, of the acetyl-serine peptides obtained by degradation of acetyl-chymotrypsin, the two which have N-terminal serine residues are N-acetyl-compounds, whereas those with other N-terminal residues are O-acetyl-compounds.

At the outset of our work it was hoped that it might afford a crucial test of the oxazoline hypothesis concerning the nature of the reactive serine in chymotrypsin and other, similar, esterases[6], since it was thought that it was only by the hydrolysis of an N-acetyl-oxazoline that N-acetyl-serine peptides

could arise. However, direct experiment[5] has shown that at *p*H 7·8 and 37°, conditions obtaining in the second stage of the enzymic degradation of acetyl-chymotrypsin[1], the O-acetyl-tripeptide (II), is converted, within 9 hr., into the N-acetyl-tripeptide (I), and that the N-acetyl-O-peptide (III) is broken down into aspartic acid and the N-acetyl-tripeptide (I). In consequence of these findings, our conclusions concerning the structures of the acetyl-serine peptides obtained from acetyl-chymotrypsin can be explained equally well on the basis of the oxazoline[6] and histidine-activation[7] hypotheses concerning the reactive serine. Thus, on the basis of the oxazoline hypothesis, the tautomeric acetylated oxazoline may be expected to break down, on hydrolysis, to give, initially, both N-acetyl-O-peptides, such as (III), and O-acetyl-N-peptides, such as (IV); the former will, however, under the degradation conditions, break down further to give N-acetyl-peptides containing N-terminal serine residues, which will, in agreement with our results, be the only isolable N-acetyl-peptides. Likewise, although the histidine-activation hypothesis requires that only O-acetyl-peptides should be formed initially, those containing N-terminal serine will isomerize, under the degradation conditions, to N-acetyl-peptides, again in agreement with our findings. Our results cannot, therefore, be used to discriminate between the two hypotheses; for this purpose, different methods of approach are required, and work of this kind is in progress in both our laboratories.

We thank the University of Exeter for the award of an Imperial Chemical Industries Fellowship, and Shell Research, Ltd., for a grant, which enabled one of us (L. B.) to take part in this work.

LEO BENOITON
H. N. RYDON

Washington Singer Laboratories,
The University,
Exeter.

R. A. OOSTERBAAN
M. E. VAN ADRICHEM
J. A. COHEN

Medical Biological Laboratory,
RVO-TNO,
Rijswijk Z.H.,
The Netherlands.

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## Ultra-violet Absorption Spectra of Proteins in Deuterium Oxide

THE absorption spectra of proteins are characterized by two absorptions in the ultra-violet region, one at 2800 and the other near 2000 Å. The former has been ascribed to the phenol[1,2], indole[3] and phenyl groups[4] of tyrosine, tryptophan and phenylalanine. The other absorption at 2000 Å. may be attributed to peptide bonds[4]. Since the absorption at 2800 Å. is due to the chromophoric groups of aromatic amino-acids, this absorption is absent in proteins, such as clupein, which do not contain aromatic amino-acid, and they have only one absorption near 2000 Å.

The contribution of aliphatic amino-acid to the ultra-violet absorption spectrum of protein solutions has not been thoroughly investigated because they do not show conspicuous absorption, except that near 2000 Å. which may be due to the carbonyl group in the COOH group[5,6].

The purpose of this work was to study the ultra-violet absorption of proteins in deuterium oxide as well as in water and to examine whether the deuteration of proteins gives rise to a change in the absorption spectrum in this wave-length region. The absorption spectra of cytochrome *c* and myoglobin, which were purified from horse heart muscle, and also ovalbumin and clupein sulphate are illustrated in the figures. According to Fig. 1 (1), cytochrome *c* has distinct absorption in deuterium oxide at 2650 Å. while it is absent in water. Although the absorption at 2800 Å. is almost hidden behind that at 2650 Å., there is still a shoulder at 2800 Å. which indicates that the electronic transition of chromophoric groups of aromatic amino-acids is not affected by deuteration and that the unknown absorption might arise from entirely different electronic transition. As is obvious from Fig. 1 (2), ovalbumin has a double-peaked absorption curve in deuterium oxide, one maximum at 2650 and the other at 2800 Å. Distinct separation of both absorptions indicates more clearly that they have different origins. Myoglobin (Fig. 1 (3)) also shows absorption at 2650 Å. in deuterium oxide, however, to a lesser extent.

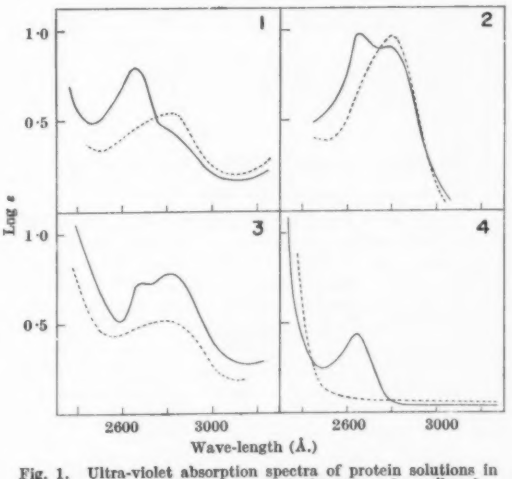


Fig. 1. Ultra-violet absorption spectra of protein solutions in deuterium oxide and water. 1, Cytochrome *c*; 2, ovalbumin; 3, myoglobin; 4, clupein, respectively. The ordinates show the relative absorption coefficients

Clupein is a protein which consists only of aliphatic amino-acids[7]. Accordingly the absorption at 2800 Å. which is characteristic of aromatic amino-acids is absent in this protein, as is shown in Fig. 1 (4) (broken line). However, this protein also shows absorption at 2650 Å. when it is dissolved in deuterium oxide, as is indicated by the solid line of Fig. 1 (4). This may be the evidence that the absorption at 2650 Å. in deuterium oxide has nothing to do with the electronic excitation of chromophores of aromatic amino-acids.

Further analyses with peptides indicate that the absorption cannot be attributed to the deuteration of the peptide bond and that it arises from the constituent simple amino-acids. Measurements with amino-acids revealed that valine, norvaline, aspartic acid, leucine, glycine and histidine showed strong absorption at 2650 Å. in deuterium oxide, but glutamic acid, alanine, aminobutyric acid and arginine and cystine did not show well-defined absorption. Measurement of tyrosine was obscured by the presence of strong absorption at 2750 Å., and I could not observe the appearance of the absorption at 2650 Å. These results indicate that some of the aliphatic amino-acids make a major contribution to the absorption of protein at 2650 Å. in deuterium oxide, rather than aromatic amino-acid, a phenomenon which has not been observed in water.

Although I have not been able to attribute this absorption to the electronic configuration of particular groups it is not likely to be due to the deuteration of dissociable groups, such as COOH, CONH or $NH_2$, which are capable of exchanging a hydrogen atom with deuterium atom very quickly, but may be due to the partial deuteration of non-polar groups such as hydrocarbon in α or β positions.

I am indebted to Dr. H. Tamiya for his continuous encouragement.

Shiro Takashima

Department of Biochemistry and Biophysics,
University of Tokyo.

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## Structure of the Antibiotics *PA114B-1* and *PA114B-3*

We wish to report the partial structures of *PA114B-1* and *PA114B-3*, members of the *PA114* group of synergistic antibiotics obtained from a strain of *Streptomyces*[1]. Other reported synergistic antibiotic groups include streptogramin[2], staphylomycin[3], antibiotic *E129*[4], and mikamycin[5]. Bioautographic comparisons by us[6] and others[4,5] have indicated identities and close similarities between various members of the groups. Table 1 summarizes these comparisons and embodies a proposed universal system of designations.

Table 1. Classification of Synergistic Antibiotics

| Type | Specific compound | Synonyms |
|---|---|---|
| *A* | *A1* | *PA114A-1*<br>*E129* Factor *A*<br>Mikamycin *A*<br>Streptogramin, main component<br>Staphylomycin *MI* |
| | *A2* | Staphylomycin *MII* |
| *B* | *B1* | *PA114B-1*<br>*E129* Factor *B*<br>Mikamycin *B* |
| | *B2* | Staphylomycin *S* |
| | *B3* | *PA114B-3* |
| | Uncertain | Streptogramin, minor component |

Antibiotics of the *A* group are neutral compounds giving a green ferric chloride test and a red colour with Ehrlich's reagent. The *B* antibiotics behave as weak acids, are negative to Ehrlich's reagent, and give a red ferric chloride test. One *A* antibiotic plus one *B* antibiotic constitute a synergistic pair having an antibacterial potency much higher than that of either component measured alone.

The characteristic properties of *PA114B-1* and *PA114B-3* clearly differentiate these antibiotics from staphylomycin factor *S* (Table 2).

Sealed-tube hydrolysis of *PA114B-1* with 6 *N* hydrochloric acid at 120° was followed by two-dimensional chromatography. Butanol/acetic acid/water (5 : 1 : 4) and butanol/water/methylethyl ketone/diethylamine (10 : 10 : 5 : 1) gave the best separation of components. One ferric chloride-positive and seven ninhydrin-positive spots were observed. Displacement chromatography of a hydrolysate on 'Dowex 50' (partial separation) and quantitative paper chromatography using the butanol/acetic acid/water system was followed by aqueous elution. Vigorous hydrolysis (3 days, 120°) indicated that none of the compounds was a peptide. The following components were identified on the basis of $R_F$ values, ninhydrin colours, specific rotations, and ultra-violet and infra-red spectra: L-threonine (Thr), L-phenylglycine (PhGly), L-proline (Pro), sarcosine (Sar), and D-α-aminobutyric acid (But). The optical configurations were confirmed by chromatography of a hydrolysate treated with D-amino-acid oxidase; only the spot corresponding to α-aminobutyric acid was lost.

From the initial aqueous wash of the 'Dowex 50' column chromatography an acidic component was isolated and identified as 3-hydroxy-picolinic acid (HyPic) on the basis of spectra and mixed melting point with an authentic sample.

Two additional ninhydrin-positive components were isolated from the paper chromatograms. Their characteristics are unlike those of any previously reported compounds. One of these unique amino-acids exhibited a yellow ninhydrin colour, a negative isatin test, and was displaced early from the 'Dowex 50' column. It had no characteristic ultra-violet absorption; analysis indicated no C-methyl, *N*-methyl, or alkoxyl. The properties are consistent with 5-hydroxymethylhydroxyproline-type compound (HMHyPro). Calculated for $C_6H_{11}O_4N$: C, 44·71; H, 6·88; N, 8·69; found: C, 44·98; H, 6·99; N, 8·58.

The second unique component gave a typical purple ninhydrin colour and exhibited ultra-violet and infra-red spectra indicative of an aromatic (dimethyl) amino-derivative of an α-amino-acid. These properties, together with elemental and group analyses, indicate a dimethylamino (methyl) phenylalanine-type structure (DMAMPhe). Direct concentration of an acid hydrolysate of *PA114B-1* led to the isolation of this amino-acid as its dihydrochloride: m.p. 207°, $[\alpha]_D + 33°$. Calc. for $C_{12}H_{20}N_2O_2Cl_2$: C, 48·82; H, 6·83; N, 9·49; Cl, 24·02, C-$CH_3$(1), 5·0; N-$CH_3$(2), 19·7; found: C, 48·80; H, 6·89; N, 9·41; Cl, 22·93; C-$CH_3$, 1·52; N-$CH_3$, 20·18.

Treatment of *PA114B-1* with 0·1 *N* sodium hydroxide for 30 min. at 25° and extraction at *p*H 3 with chloroform led to the isolation of a dibasic acid. Complete acid hydrolysis indicated that it contained all the components of *PA114B-1*. Hydrazinolysis of this new acid indicated that phenylglycine was C-terminal; *PA114B-1* contains no C-terminal amino-

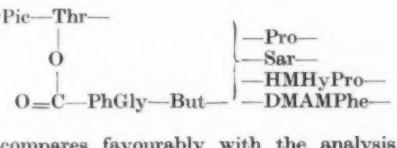
Table 2. PROPERTIES OF *PA114B*-1, *PA114B*-3 AND STAPHYLOMYCIN *S*

| | *PA114B*-1 | *PA114B*-3 | Staphylomycin factor *S* |
|---|---|---|---|
| Melting point | 265° | 208° | 240° |
| Optical activity, $[\alpha]_D^{25°}$ | −59·7° | −37·2° | −28·0° |
| Elemental analysis C | 62·07 | 62·77 | 62·80 |
| H | 6·21 | 6·52 | 6·57 |
| N | 12·77 | 12·61 | 11·46 |
| Ultra-violet maxima (MeOH) | 260 mμ (*E*(1% 1 cm.) = 217)<br>305 mμ (*E*(1% 1 cm.) = 105) | 258 mμ (*E*(1% 1 cm.) = 204)<br>305 mμ (*E*(1% 1 cm.) = 95) | 207 mμ (*E*(1% 1 cm.) = 590)<br>304 mμ (*E*(1% 1 cm.) = 86) |
| Neutral equivalent | 1,000 | 1,070 | 790 |
| *pKa* (MeOH) | 8·5 | | 9·0 |

acid. The generation of a terminal group by mild alkaline hydrolysis indicated the presence of a peptide chain closed by means of a lactone linkage—the carboxyl of phenylglycine to the hydroxyl of one of the hydroxyamino-acids. The similarity between *PA114B*-1 and staphylomycin factor *S* (ref. 7) makes a phenylglycine to threonine linkage, as in the latter, probable. Hydroxypicolinic acid, being incapable of forming a peptide via the nitrogen, must be N-terminal.

Partial hydrolysis of *PA114B*-1 (1 *N* hydrochloric acid, 37°, 1 week) and paper chromatography led to the isolation of two fluorescent peptides. After complete hydrolysis and two-dimensional chromatography on the systems described above, one of these peptides was found to have all the components of *PA114B*-1 except phenylglycine, confirming its C-terminal nature. The other peptide lacked only phenylglycine and α-aminobutyric acid.

At this stage we postulate the structure of *PA114B*-1 as:




This compares favourably with the analysis of Table 2 (calc. for $C_{48}H_{61}N_9O_{12}$: C, 60·33; H, 6·60; N, 13·11).

*PA114B*-3[6], a minor antibiotic of the *PA114* complex, contains all the components of *PA114B*-1 except sarcosine. A new component (negative ninhydrin and ferric chloride tests) is also present; a positive *p*-nitrobenzoyl chloride test[8] indicates that it is probably an N-methyl amino-acid.

Work on the structures of *PA114B*-1 and *PA114B*-3 are continuing.

D. C. HOBBS
W. D. CELMER

Biochemical Research Department,
Chas. Pfizer and Co., Inc.,
Research Laboratories,
Groton,
Connecticut.

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## Presence of Immunologically Active Fragments after Proteolytic Degradation of Human γ-Globulin

ACCORDING to Lapresle, Kaminski and Tanner[1], degradation of human serum albumin with trypsin or chymotrypsin yields several components with retained ability to give precipitation reactions with anti-human albumin antibodies. One of these components has been isolated by column electrophoresis in an agar medium and partially characterized by ultracentrifugation[2]. Porter[3] isolated and characterized a similar component after chymotryptic hydrolysis of bovine serum albumin. After degradation of rabbit γ-globulin with papain, Porter also isolated a component showing inhibitory properties when added to a rabbit γ-globulin–anti-γ-globulin system[4].

We have carried out immunological analyses of human γ-globulin after degradation with trypsin and chymotrypsin, respectively. Immunologically pure γ-globulin (as shown with anti-human plasma sera in immune electrophoresis), supplied by AB Kabi, Sweden, was used. Amounts of 0·1–1·0 gm. γ-globulin were hydrolysed at *p*H 8·2 and 25° C. with commercial preparations of crystalline enzymes, using enzyme/substrate ratios of 1 : 50. After 24 hr. the reaction was interrupted by the addition of di*iso*propyl fluorophosphate[5]. The hydrolysates were analysed by means of immune electrophoresis[6].

The results of these analyses are shown in Fig. 1. After tryptic hydrolysis, the γ-globulin gave rise to two crossing lines when analysed with anti-γ-globulin immune sera (Behringwerke, Germany). The chymotryptic hydrolysate showed by similar analysis a dense precipitate which resembled the regular γ-globulin line. In addition two other lines

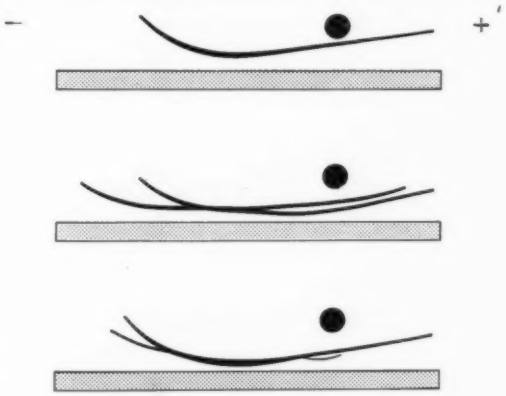


Fig. 1. Immune electrophoresis of human γ-globulin (top basin), γ-globulin degraded by trypsin (middle basin) and γ-globulin degraded by chymotrypsin (lower basin). Immune serum: anti-human γ-globulin

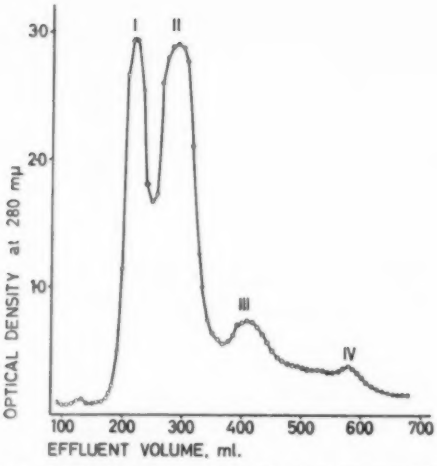


Fig. 2. Gel filtration of a tryptic hydrolysate of human γ-globulin on 'Sephadex *G*-75'. Eluant: 0·05 *M* sodium chloride

were formed, one farther out in the γ-globulin region and the other near the starting basin. All three lines were interconnected.

Preliminary experiments to separate the breakdown products demonstrated immunologically were carried out by the gel filtration method described by Porath and Flodin[7]. A dextran gel with a water regain of 4·9 ('Sephadex *G*-50', manufactured by AB Pharmacia, Sweden) was first tried. It was found, however, that more than 80 per cent of the degraded γ-globulin passed through the column without any retention. Therefore a gel with a lower degree of cross-linking was tried ('Sephadex *G*-75' with a water regain of 8·0). The column (4 cm. × 85 cm.) was equilibrated with 0·05 *M* sodium chloride, and the uniformity of the packing was tested by passing a hæmoglobin solution through the column. 500 mgm. of a tryptic hydrolysate of γ-globulin in 5 ml. of 0·05 *M* sodium chloride was then put on the column and eluted with a rate of flow of 20 ml. per hr. The effluent was collected in 7–8 ml. fractions, and the optical density of each fraction at 280 mμ was determined. The resulting curve is seen in Fig. 2. Immune electrophoretic analyses were carried out on fractions I–III, and the results are shown in Fig. 3. Fraction I showed one dense precipitate with its maximum mainly in the $\beta_2$- but extending into the γ-globulin region, where a confluent precipitate, farther out in the γ-globulin region, joined it. Fraction II showed two crossing lines, one with its maximum in the β-globulin region and the other with a maximum corresponding to slow-moving γ-globulin. With fraction III only a very faint precipitate was formed in the $\beta_2$-globulin region. Analogous results were obtained by gel filtration of a chymotryptic hydrolysate of γ-globulin (Cederblad, G., Hanson, L. Å., and Johansson, B. G., unpublished work).

From these results it can be concluded that normal human γ-globulin contains different antigenic groupings, with the ability to react with anti-γ-globulin antibodies. Corresponding findings concerning pathological γ-globulins have been presented by Scheidegger and Buzzi[8].

Further work on the isolation and characterization of immunologically active degradation products from immune globulins is in progress.

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L. Å. Hanson

Department of Bacteriology,

B. G. Johansson

Department of Medical Biochemistry,
University of Gothenburg.
April 11.

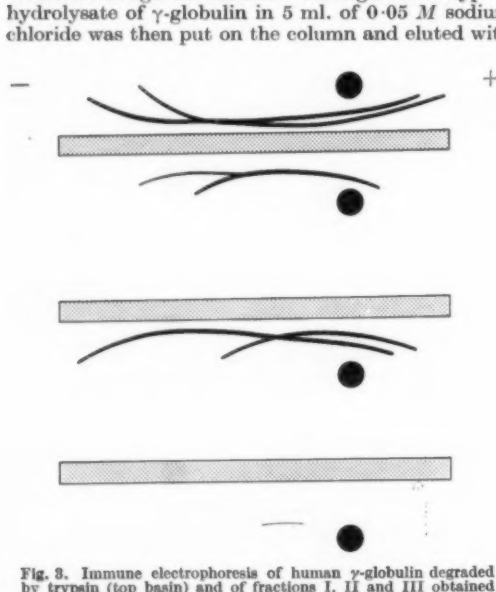


Fig. 3. Immune electrophoresis of human γ-globulin degraded by trypsin (top basin) and of fractions I, II and III obtained by gel filtration of this degraded γ-globulin. Immune serum: anti-human γ-globulin

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## Starch-Gel Electrophoresis of Wheat Proteins

It has been claimed that starch-gel electrophoresis may have a higher resolving power than that of the Tiselius apparatus for serum proteins[1]. In spite of its potential value, there have been no reports in the literature of the application of this technique to the study of wheat proteins. Wheat gluten has been separated into at least six components by Jones *et al.*[2], using the Tiselius apparatus, and Zentner[3] has reported the presence of seven proteins, using continuous paper electrophoresis. The present communication describes some preliminary experiments in which starch-gel electrophoresis has been used to examine the proteins of the gluten complex of wheat.

10 gm. of wet gluten were prepared from wheat flour by hand kneading in distilled water and were dispersed in 200 ml. *N*/100 acetic acid in a high-speed

stirrer, fitted with sharp steel blades, in 5 min. After centrifuging for 30 min. at 2,000 r.p.m., the liquid was subjected to starch-gel electrophoresis. Both horizontal and vertical[4] techniques were used. Connaught starch was used, 12·2 gm. mixed with 100 ml. of an aluminium lactate buffer[2]. The sample slot (1–2 mm. wide) was filled with protein solution mixed with starch granules, but in later work the starch was omitted without affecting the quality of the patterns. Runs were normally made for 2–4 hr. at a potential gradient of 4–6 V. cm.$^{-1}$. The gels were sliced and the cut surfaces stained with naphthalene black 10*B*.

Centrifugation at 2,000 r.p.m. does not remove colloidal particles, which migrate into the gel for several mm., and tend to obscure some of the slower-moving bands. They stain strongly, but can be distinguished from a true band by the characteristic irregular appearance. Under a low-power microscope the 'band' can be seen to consist of dyed particles. When a dispersion was cleared by ultracentrifugation at 20,000*g*, the 'band' no longer appeared on electrophoresis.

Table 1

| Mobility (cm.$^2$ V.$^{-1}$ sec.$^{-1}$) | Intensity of band | | | |
|---|---|---|---|---|
| | Bison | Flamingo | Rescue | Witchita |
| 2·6 | *m* | ? | ? | *m* |
| 3·5 | *s* | *m* | ? | *m* |
| 4·2 | *s* | *s* | *s* | *s* |
| 6·4 | *f* | *m* | *m* | *f* |
| 8·3 | *m* | *s* | *m* | *m* |
| 9·2 | *f* | *m* | *m* | *f* |
| 10·2 | *vf* | *f* | *f* | *f* |
| 12·7 | *vf* | *f* | *f* | *f* |

*vf*, very faint; *m*, medium; *s*, strong; ?, partially obscured.

Table 1 gives an example of electrophoresis patterns obtained on a four-channel vertical apparatus[4] for glutens from four wheat varieties of widely differing baking characteristics (Table 2). It is interesting to note that each of the four varieties examined shows patterns of similar electrophoretic mobilities, and the patterns differ only in the distribution of intensities. It should be pointed out that the presence of eight distinct and separate bands in the electrophoresis patterns does not necessarily prove that eight different proteins are present, since some of the bands could conceivably arise as a result of complex formation.

Table 2

| Wheat | Source | Type | Baking strength |
|---|---|---|---|
| Bison | U.S.A. | Hard red winter | Strong |
| Flamingo | England | White winter | Very weak |
| Rescue | U.S.A. | Hard red spring | Weak |
| Witchita | U.S.A. | Hard red winter | Medium |

The pattern of mobilities quoted in Table 1 is typical of the results of a large number of experiments, in all of which at least eight distinct bands have been found, but variations of the order of 20 per cent in mobility are found when comparing individual runs. This is probably due principally to the difficulty in preparing gels of precisely reproducible properties. The mobilities reported here are higher than those obtained for wheat proteins by Jones *et al.*[2] at 1° C. The highest value obtained by them was $6·5 \times 10^{-5}$ cm.$^2$ V.$^{-1}$ sec.$^{-1}$. Our experiments were carried out at approximately 25° C., and the increase in conductivity, due to the use of a higher temperature, would be enough to account for the increased mobilities, since the presence of starch up to concentrations of 12·5 per cent does not significantly affect the electrical conductivity of salt solutions (Collison, R., private communication).

The work reported here demonstrates that starch-gel electrophoresis is a valuable method for investigating wheat proteins, as complete resolution of the eight bands reported was obtained. Further studies of the protein components in wheats of different origin are in hand, using this method in conjunction with column chromatography and immuno-techniques.

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G. A. H. Elton
J. A. D. Ewart
British Baking Industries Research Association,
Chorleywood, Herts.

April 25.

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## Activity of Bovine Pancreatic Ribonuclease *A* in Mixed Solvent Systems

It has been shown that bovine pancreatic ribonuclease may be dissolved in organic solvents with full recovery of the activity after removal of the solvent[1]. We have found that ribonuclease *A* retains its activity in the presence of high concentrations of many organic solvents and have utilized this property for investigating its mode of action. If the solvent used is methanol then the products of the action of ribonuclease on cytidine-2′,3′-phosphate are cytidine-3′-phosphate and cytidine-3′-phosphate methyl ester as previously observed by Heppel and Whitfeld[2]. As the mole fraction of methanol is raised, methanolysis increases and hydrolysis decreases as shown in Fig. 1. This behaviour is consistent with competition between methanol and water for a site on the enzyme.

The effect of solvents on enzyme-catalysed reactions is due to many causes including shifts in $pK$'s of buffers, substrate and protein as well as direct effects on the binding and catalysis. Thus solvent effects have contributed little to our understanding of enzyme catalysis. However, for ribonuclease it is possible to observe simultaneously the effect of addition of solvent on methanolysis and hydrolysis. This has been achieved by keeping a constant ratio of water : methanol while varying the concentration of another, inert, organic solvent (dioxane, formamide and dimethyl formamide). Under these conditions the solvent will have widespread effects but those involving the cyclic phosphate probably do not influence the relative rates of hydrolysis and methanolysis. Thus any changes in the relative rates which are observed may be attributed to the effects of the solvent on the interaction of water or alcohol with the protein. In Fig. 2 are shown the effects of dioxane and formamide on the ratio of the initial rates of esterification and hydrolysis. While formamide has little effect on this ratio, hydrolysis is very much favoured by increasing amounts of dioxane. This effect is not due to alteration of $pK$'s since the ratio esterification : hydrolysis was found to be substantially independent of $p$H when this was varied over a range covering any likely $pK$ change. It is

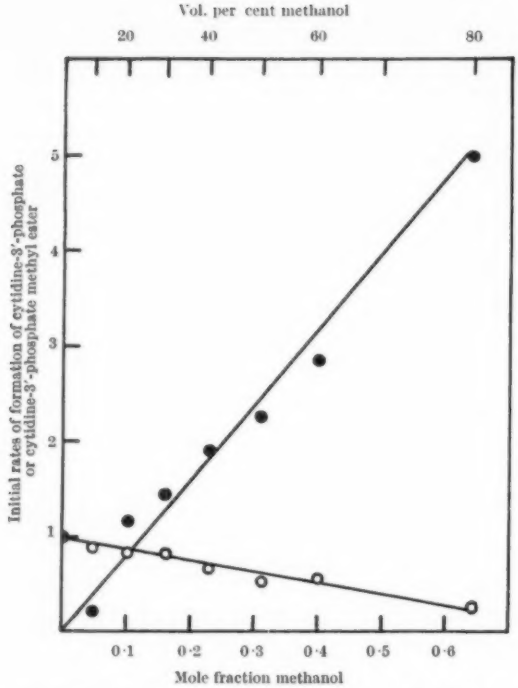


Fig. 1. The effect of methanol on the initial rates of formation of cytidine-3′-phosphate methyl ester (●) and cytidine-3′-phosphate (○). Rates are relative to hydrolysis of cytidine-2′,3′-phosphate in absence of methanol. Cytidine-2′,3′-phosphate, 0·05 *M* in citrate buffer at *p*H 6·9. Temperature, 25° C.

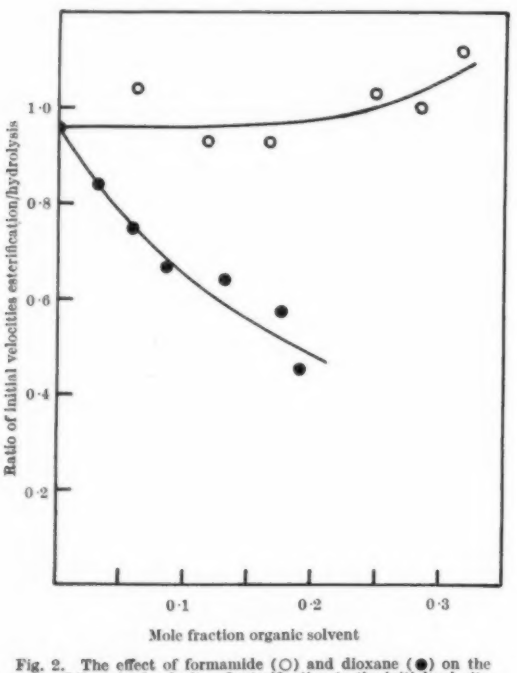


Fig. 2. The effect of formamide (○) and dioxane (●) on the ratio of the initial velocity of esterification to the initial velocity of hydrolysis of cytidine-2′,3′-phosphate. Ratio water : methanol constant and equal 3 : 1 v/v

evident that there is a site on the enzyme surface which binds either water or methanol. The superiority of methanol as an attacking reagent (Fig. 1) and the effect of dioxane suggest that there is a Van der Waals interaction between methanol and the protein. Dioxane favours hydrolysis relative to methanolysis by interfering with the Van der Waals interaction.

It can be seen that considerable information can be obtained by measuring the activity of enzymes in mixed solvent systems. By investigating the effect of organic solvents on the *p*H activity curves using buffers of both types of charge, it should be possible to determine the type of charge of the ionizing groups controlling the enzyme activity.

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Dorothy Findlay
A. P. Mathias
B. R. Rabin

Department of Biochemistry,
University College,
London.

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## Action of Tetrakis(Hydroxymethyl)Phosphonium Chloride on Wool

In an investigation of the action of tetrakis(hydroxymethyl)phosphonium chloride (THPC) on wool the following interesting observations have been made.

When wool treated with tetrakis(hydroxymethyl)phosphonium chloride is dyed together with a sample of untreated wool with acid or premetallized dyes, the treated wool absorbs much more dye than the untreated. Uptake of the basic dye methylene blue is, however, less for the treated than for the untreated wool. The addition of tetrakis(hydroxymethyl)phosphonium chloride to the dye liquor greatly accelerates the rate of dye absorption.

When wool fibres, stretched 40 per cent, are boiled for various times in tetrakis(hydroxymethyl)phosphonium chloride solution (2 per cent) and afterwards relaxed for 1 hr. in boiling water, the results shown in Fig. 1 are obtained. It is evident that the amount of

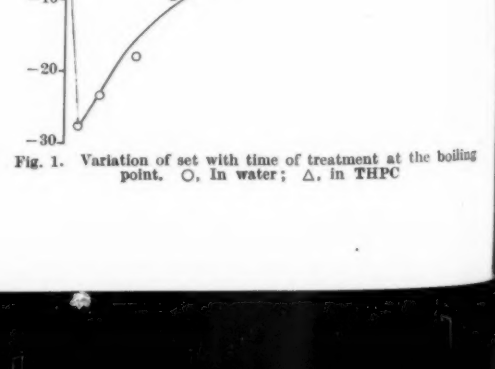


Fig. 1. Variation of set with time of treatment at the boiling point. ○, In water; △, in THPC

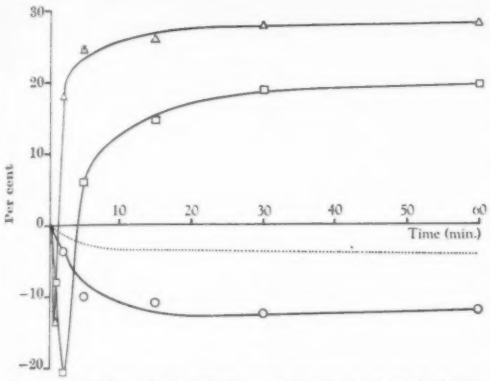


Fig. 2. Variation of set with time of treatment at the boiling point. ...., Deaminated, iodinated and potassium cyanide-treated fibres in water; △, iodinated fibres in THPC; □, deaminated fibres in THPC; ○, potassium cyanide-treated fibres in THPC

set retained by the fibres after they have been afterwards boiled freely is much greater than after a similar treatment of stretched fibres in boiling water. The effect of tetrakis(hydroxymethyl)phosphonium chloride depends on the strength of the solution, but is marked even at a concentration of 0·1 per cent. In dilute solutions the supercontraction characteristic of stretched fibres in boiling water is observed. Typical results are shown in Table 1.

Table 1

| Concentration of THPC solution (per cent) | Set (+ve sign) or supercontraction (−ve sign) after relaxing the fibres, treated in the stretched state for 2 min. | 120 min. |
|---|---|---|
| 0·1 | −17·8 | 28·6 |
| 0·5 | +4·9 | 22·9 |
| 1·0 | 13·0 | 22·5 |
| 2·0 | 17·1 | 24·5 |

It is not possible to set stretched fibres which have been previously iodinated or deaminated in boiling water, but these materials are readily set in a solution of tetrakis(hydroxymethyl)phosphonium chloride (2 per cent). It is not, however, possible to set stretched fibres which have been previously treated with potassium cyanide, either in water or tetrakis (hydroxymethyl)phosphonium chloride solutions. These results are shown in Fig. 2.

When slack fibres are boiled in a solution of tetrakis(hydroxymethyl)phosphonium chloride (1 per cent) for 1½ min. at *p*H 7, supercontraction occurs to the extent of 38 per cent.

The reaction of tetrakis(hydroxymethyl)phosphonium chloride with wool is no doubt complex; but at present it is considered to involve reduction of the disulphide bond as the primary process, accompanied by the absorption of the phosphonium ion, thus increasing the number of positive sites in the fibre. Work on this topic is proceeding and will be reported in full elsewhere.

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L. S. Bajpai
C. S. Whewell
J. M. Woodhouse

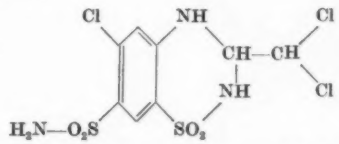
Department of Textile Industries,
The University,
Leeds, 2.

## PHYSIOLOGY

### A Potent New Benzothiadiazine Diuretic

Since the discovery of chlorothiazide by Beyer *et al.*[1], a number of chemically related compounds have been shown to possess similar natriuretic and chloruretic actions[2–4]. Some of these agents, notably 3,4-dihydrochlorothiazide and its derivatives, differ from chlorothiazide primarily in a greater milligram potency, which may be associated with a longer duration of action[5]. These agents also inhibit carbonic anhydrase to a lesser extent than the unsaturated analogues, resulting in a lower urinary *p*H and generally a greater chloruretic response[5,6].

In an effort to determine how further structural modifications of the chlorothiazide nucleus might alter the renal actions, we have undertaken an investigation of derivatives substituted in the 3 position of the dihydro analogue[7]. This is a preliminary report of pharmacological studies on one of these compounds, trichlormethiazide (*NAQUA*™ is the Schering brand of trichlormethiazide), which has been characterized by marked diuretic activity at very low doses. The chemical formula of this agent is as follows:




3-dichloromethyl-6-chloro-7-sulphamyl-3,4-dihydro-1,2,4 - benzothiadiazine - 1,1 - dioxide (trichlormethiazide).

The effect of trichlormethiazide on urine and electrolyte excretion was studied in anæsthetized dogs in which the ureters were cannulated and total urine was collected every 5 min. Urine samples were analysed for sodium, potassium and chloride. Typical results of intravenous administration of 1·0 μgm./kgm. are presented in Table 1.

Table 1. Effect of Trichlormethiazide on Urine Output and Electrolyte Excretion in an Anæsthetized Dog

| Time (min.) | Urine (ml./min.) | Sodium | Potassium | Chloride |
|---|---|---|---|---|
| | | Microequivalents per min. | | |
| −15 | 0·26 | 98 | 43 | 68 |
| −10 | 0·26 | 101 | 39 | 65 |
| −5 | 0·24 | 100 | 31 | 62 |
| 0 | 1·0 μgm./kgm. trichlormethiazide (intravenous) | | | |
| 5 | 0·28 | 112 | 32 | 68 |
| 10 | 0·32 | 136 | 34 | 85 |
| 15 | 0·34 | 134 | 30 | 98 |
| 20 | 0·32 | 128 | 30 | 96 |
| 25 | 0·30 | 122 | 30 | 88 |
| 30 | 0·28 | 126 | 31 | 82 |
| 35 | 0·24 | 97 | 25 | 67 |

These results demonstrate that a significant natriuretic and chloruretic response can be elicited at doses as low as 1·0 μgm./kgm. and that the sodium and chloride increases are equimolar. Potassium excretion increased slightly but considerably less than sodium and chloride. The maximum natriuretic response occurred approximately 10 min. after administration. Duration of action was 30 min.

The relative activities of trichlormethiazide in terms of chlorothiazide, and trichlormethiazide in terms of 3,4-dihydrochlorothiazide, by the oral route have been determined in unanæsthetized dogs using a modified cross-over design (32 animals for each

assay). Dose-levels (2) and compounds (2) were both crossed over on two experimental days set one week apart. The doses were selected on the basis of previous experimental data to elicit approximately the same natriuretic response. Urine and sodium excretion-rates were determined for a 5-hr. period after treatment. Relative activities of trichlormethiazide were obtained by comparing the dose-response curves and analysing the results by statistical methods. The results are summarized in Table 2.

Table 2. Comparative Activity of Three Thiazide Diuretics on Urine Output and Sodium Excretion in Dogs

| Compound | Dose μgm./kgm. | Urine output ml./5 hr. | Urine output Relative activity | Sodium excretion m.eq./5 hr. | Sodium excretion Relative activity |
|---|---|---|---|---|---|
| Chlorothiazide versus trichlormethiazide | | | | | |
| *CT* | 500 | 54 | 1·0 | 14·3 | 1·0 |
| *CT* | 3,000 | 73 | | 16·6 | |
| *TCM* | 15 | 54 | 58·9 | 11·4 | 72·0 |
| *TCM* | 90 | 97 | (25·2–168·0)* | 27·2 | † |
| 3,4-Dihydrochlorothiazide versus trichlormethiazide | | | | | |
| *HCT* | 100 | 50 | 1·0 | 12·8 | 1·0 |
| *HCT* | 600 | 84 | | 18·4 | |
| *TCM* | 15 | 62 | 7·9 | 16·6 | 13·6 |
| *TCM* | 90 | 79 | (7·1–9·4)* | 19·6 | (6·5–34·0)* |

* 95 per cent confidence limits.
† Confidence limits could not be calculated since analysis showed significant non-parallelism between curves at $P$ 0·01.

On a dose basis, trichlormethiazide was found to be 72·0 times more active than chlorothiazide and 13·6 times more active than 3,4-dihydrochlorothiazide in promoting the excretion of sodium. Potency values for urine output were 58·9 and 7·9, respectively. Average urine output in the same animals, untreated, was found to be 30–40 ml./5 hr. while control sodium excretion averaged 4–8 m.equiv./5 hr. These findings suggest that the minimally effective oral dose of trichlormethiazide is less than 15 μgm./kgm., and that this agent, like chlorothiazide and 3,4-dihydrochlorothiazide, is well absorbed through the gastrointestinal tract.

Richard M. Taylor
Martin M. Winbury

Department of Pharmacology,
Scientific Research Division,
Schering Corporation,
Bloomfield, New Jersey.

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## Effect of Cocaine on the Disposition of Noradrenaline labelled with Tritium

Cocaine potentiates the responses of sympathetically innervated organs to adrenaline, noradrenaline and sympathetic nerve stimulation, and several explanations have been offered for this. Cannon and Rosenblueth[1] suggested that cocaine might act by preventing the destruction of injected adrenaline, or of transmitter substance released from adrenergic nerves, and Torda[2] and Trendelenburg[3] obtained evidence in support of this hypothesis. Macmillan[4] suggested that cocaine might prevent the uptake of catecholamines into tissue stores, and block their release from these stores, but his evidence was only indirect.

O-methylation is a major route for the inactivation of circulating catecholamines[5], and, more recently, binding by many tissues has been shown to be important for the inactivation of circulating adrenaline[6] and noradrenaline[7]. This communication describes the influence of cocaine on the uptake into tissues of noradrenaline labelled with tritium, and the formation of $^3$H-normetanephrine by O-methylation.

Male cats (1·7–3·6 kgm.) were anæsthetized with pentobarbitone sodium (35 mgm./kgm.) intraperitoneally and a tracheostomy performed. The right femoral artery was cannulated for recording the blood-pressure and the left femoral artery for the withdrawal of blood samples. Cocaine (5 mgm./kgm.), dissolved in 5 ml. 0·9 per cent (w/v) sodium chloride solution, was injected intravenously over a period of 5–10 min. When the blood-pressure had returned to a steady level, *dl*-β-$^3$H-noradrenaline (1·72 mc./mgm.) was injected intravenously (dose, 25 μgm./kgm.) within 5–10 sec. Blood samples were withdrawn periodically and the animals were decapitated 1 hr. after the injection of noradrenaline. Adrenal glands, heart, liver, abdominal wall muscle and spleen were removed immediately, homogenized and analysed for $^3$H-noradrenaline and $^3$H-normetanephrine[8].

Table 1. Effect of Cocaine on the Tissue Content of $^3$H-Noradrenaline and $^3$H-Normetanephrine

Results are expressed in mμgm. $^3$H-noradrenaline or $^3$H-normetanephrine per gm. of tissue (mean value ± *S.E.*), and were obtained with seven untreated and four cocaine-treated cats (for details of dosage see text). Cats were injected intravenously with $^3$H-noradrenaline (25 μgm./kgm.)

| Tissue | $^3$H-Noradrenaline Untreated | $^3$H-Noradrenaline Cocaine-treated | $^3$H-Normetanephrine Untreated | $^3$H-Normetanephrine Cocaine-treated |
|---|---|---|---|---|
| Adrenal gland | 121 ± 17 | 26 ± 9·0 | 9·0 ± 1·2 | 5·5 ± 0·5 |
| Heart | 165 ± 20 | 29 ± 5·8 | 16·4 ± 3·2 | 5·2 ± 0·4 |
| Spleen | 151 ± 21 | 22·5 ± 1·9 | 17 ± 1·9 | 7·1 ± 0·5 |
| Liver | 18·7 ± 3·9 | 8·0 ± 0·7 | 8·0 ± 1·1 | 6·3 ± 1·2 |
| Skeletal muscle | 4·6 ± 0·7 | 3·0 ± 0·5 | 5·2 ± 1·1 | 8·1 ± 0·7 |

The effect of cocaine on the concentrations of $^3$H-noradrenaline and $^3$H-normetanephrine in tissues is shown in Table 1. Adrenal glands, heart and spleen are three tissues in which uptake and retention of injected noradrenaline has been shown to be greatest[7]; in these organs administration of cocaine has resulted in a marked reduction in their content of $^3$H-noradrenaline. The quantity of $^3$H-noradrenaline in liver and skeletal muscle 1 hr. after the injection was also reduced, but to a smaller extent. The effect of cocaine on the tissue-levels of $^3$H-normetanephrine was less marked; reductions were observed in heart and spleen and an elevation in skeletal muscle. Because of its bulk, muscle accounts for most of the $^3$H-noradrenaline and $^3$H-normetanephrine remaining in the animals at the end of the experiments.

Table 2 shows the plasma-levels of $^3$H-noradrenaline and $^3$H-normetanephrine at various times after the injection of $^3$H-noradrenaline. In the cocaine-treated animals the level of $^3$H-noradrenaline exceeded the control-levels during the first 3 min. after the injection, confirming the observations of Trendelenburg[3], but in later blood samples there was no significant difference between the two groups.

These experiments show that cocaine markedly reduces the uptake of circulating noradrenaline into

Table 2. INFLUENCE OF COCAINE ON PLASMA LEVELS OF $^3$H-NORADRENALINE AND $^3$H-NORMETANEPHRINE

Results are expressed in mμgm. $^3$H-noradrenaline or $^3$H-normetanephrine per ml. of plasma (mean value ± *S.E.*)

| Time after injection (min.) | $^3$H-Noradrenaline | | $^3$H-Normetanephrine | |
|---|---|---|---|---|
| | Untreated | Cocaine-treated | Untreated | Cocaine-treated |
| 1 | 89·3 ± 9·5 | 212 ± 19 | 3·9 ± 0·5 | 5·5 ± 0·7 |
| 2 | 49·3 ± 5·8 | 89·5 ± 9·0 | 5·7 ± 1·0 | 6·4 ± 0·9 |
| 3 | 31·1 ± 3·1 | 51·7 ± 7·5 | 5·6 ± 0·7 | 8·4 ± 1·1 |
| 5 | 21·5 ± 1·7 | 25·1 ± 2·8 | 5·5 ± 0·8 | 8·3 ± 1·2 |
| 10 | 13·8 ± 1·3 | 13·3 ± 0·9 | 3·6 ± 0·5 | 4·8 ± 0·6 |
| 30 | 8·5 ± 1·4 | 7·3 ± 0·5 | 1·5 ± 0·1 | 1·9 ± 0·1 |
| 60 | 5·4 ± 0·5 | 3·9 ± 0·4 | 1·1 ± 0·2 | 0·8 ± 0·05 |

certain tissues, presumably by interfering with binding of the hormone. This interference with the uptake raises the amount of circulating catecholamine, and this is reflected in the increased plasma noradrenaline-levels observed in the early stages after an intravenous injection. Noradrenaline therefore exists in a free and active state for a longer period than in untreated animals, and these observations may be sufficient to explain the effect of cocaine in increasing the sensitivity of sympathetically innervated organs to circulating catecholamines.

It is unlikely that cocaine is acting by interfering with the enzymic inactivation of injected noradrenaline, since we have found that it does not inhibit O-methyl transferase *in vitro*. Furthermore (Table 1), cocaine has had little effect on the content of $^3$H-noradrenaline, and no significant effect on the concentration of $^3$H-normetanephrine in the liver, which is the organ having the highest concentration of catechol-O-methyl transferase[9].

We have found that reserpine also reduces the tissue content of $^3$H-noradrenaline, and elevates the plasma-levels (unpublished observations). Both reserpine[10] and cocaine[1] cause supersensitivity of sympathetically innervated organs, but reserpine releases catecholamines from tissue stores[11], whereas cocaine prevents both their release[10,12] and uptake in the tissues. Although acting by different mechanisms, both drugs produce a common end-result, that is, lower tissue concentration of administered catecholamine, elevated plasma-levels of the free hormone, and consequently supersensitivity.

L. G. WHITBY*
G. HERTTING†
J. AXELROD

Laboratory of Clinical Science,
National Institute of Mental Health,
National Institutes of Health,
Bethesda, Maryland.

* Fellow of the Rockefeller Foundation. Present address: Biochemistry Department, University of Cambridge, England.
† Visiting scientist.

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## Effect of Trypan Blue on Rat Embryos

THE teratogenic effect of trypan blue in pregnant rats has been well established[1-3]. The dye appears to be most active when injected at 8½ days of pregnancy and gives rise to a variety of abnormalities involving almost all the systems of the embryo. Of a number of chemically related azo dyes tested, trypan blue was found to be the most active teratogenic agent in rats[4] and it has also been shown to act as a teratogen in other rodents[5-7] and in chicks[8]. Three main hypotheses explaining its action have been proposed. The first assumed that a part, at least, of the dye enters the blastocyst and acts directly on the embryonic cells[9]; the second postulates a 'clogging' of the yolk sac epithelium with the large dye molecules, thus inhibiting the transfer of essential substances to the conceptus; the third suggests maternal metabolic changes resulting in the formation of substances which are responsible for the fœtal malformations.

During our own preliminary investigations into the action of trypan blue it was found that the commercial sample used was grossly impure.

A brief survey (Table 1) of the purity of various other commercial samples showed that the chief impurity was sodium chloride, which is used for the salting out of the dye during its manufacture. The actual amount of the azo dye in the samples varied from 19·8 to 85·6 per cent, and for this reason it is difficult to compare dosage-levels and to assess the results of previous workers who have used commercial sources of the dye.

Paper chromatography of the dye samples has revealed in all cases a number of chromogenic impurities, the main one being red in colour. The ratio of red impurity : trypan blue, in the various dye samples, has been determined spectrophotometrically after chromatographic separation (Table 1, col. 4). This impurity has been previously noted in trypan blue[10,11] and a similar red impurity has been found in Evans's blue (*T* 1824) (ref. 12). The latter dye is also to some extent teratogenic[4].

The main red impurity ($\lambda_{max.}$ 540 mμ in *N* hydrochloric acid) was separated from the first mother liquor obtained after the recrystallization of crude trypan blue (Grübler). A 'Solkaflock' cellulose column was used with water as the eluting agent. The mother liquor was de-ionized in a Wood's electrodialyser[12] before being applied to the column, and the separated red fraction was dried *in vacuo* and dissolved in water to give a 0·5 per cent w/v solution. The red material, which is of unknown structure, diffused slowly through a 'Cellophane' membrane and when injected into rats was quickly excreted in the urine. It therefore seems feasible to assume that the red impurity of trypan blue can enter the blastocyst.

Recrystallization of trypan blue (Grübler) twice from hot water gave a blue dye ($\lambda_{max.}$ 590 mμ in *N* hydrochloric acid) which showed negligible chloride content and only a trace of the red impurity. Further purification of this material was not possible at this time due to the restricted supply of the sample available and the large losses consequent on recrystallization. The recrystallized trypan blue did not diffuse through a 'Cellophane' membrane, and when injected into rats it was not excreted in the urine; it was dried *in vacuo* and dissolved in water to give a 1 per cent w/v solution.

Table 1. Analysis of Commercial Samples of Trypan Blue

| Source | Chloride as NaCl* | Water† | Azo dye as‡ $C_{34}H_{24}O_{14}N_6S_4Na_4$ | $E_{550}$ red impurity§ / $E_{550}$ trypan blue |
|---|---|---|---|---|
| G. T. Gurr vital stain 1 | 52·6 | 6·9 | 38·2 | 0·21 |
| G. T. Gurr vital stain 2 | 76·0 | 5·5 | 19·8 | 0·21 |
| Michrome | 76·0 | 6·7 | 19·8 | 0·18 |
| Michrome, vital stain | 13·1 | 9·5 | ¶ | ¶ |
| Gerrard | 5·3 | 13·9 | 80·4 | 0·08 |
| Grübler | 9·7 | 17·2 | 73·6 | 0·16 |
| British Drug Houses, Ltd. | 1·5 | 11·6 | 84·5 | 0·07 |
| British Drug Houses, Ltd., vital stain | 1·2 | 12·3 | 85·6 | 0·10 |
| Flatters and Garnett, Ltd. | 48·7 | 11·0 | 43·2 | 0·24 |

* By potentiometric titration. † Loss of weight after 6 hr. at 110°. ‡ By titanous chloride titration. § Measured after chromatographic separation. ¶ No value could be obtained since this dye contains another major impurity, blue in colour.

Table 2

| Substance tested | No. of rats used | Total implantations | Total resorptions | Resorptions (per cent) | External abnormalities in survivors: Normal | Abnormal |
|---|---|---|---|---|---|---|
| M.R.C. hooded rats | | | | | | |
| Control (uninjected) | 14 | 148 | 10 | 6·7 | 138 | 0 |
| 1 per cent trypan blue (Grübler) | 11 | 117 | 36 | 30·7 | 80 | 1 |
| 1 per cent recrystallized trypan blue | 12 | 144 | 18 | 12·5 | 126 | 3 |
| 1 per cent mother liquor | 14 | 148 | 46 | 31·8 | 102 | 2 |
| 0·5 per cent red impurity | 19 | 224 | 47 | 20·9 | 176 | 1 |
| Wistar rats | | | | | | |
| Control (uninjected) | 4 | 33 | 2 | 6·0 | 31 | 0 |
| 1 per cent crude trypan blue | 7 | 48 | 27 | 56·2 | 9 | 12 |

The mother liquor from the first recrystallization of trypan blue (Grübler) was also dried *in vacuo* and dissolved in water to give a 1 per cent w/v solution. This solution was rich in the red impurity, the ratio of the respective $E_{max.}$ values (cf. Table 1) of the red and blue components being 0·95 compared with 0·16 in crude trypan blue (Grübler).

For testing the effect of trypan blue M.R.C. hooded rats of our laboratory strain were used. Rats in œstrous (determined by the vaginal smear) were mated overnight and pregnancy was timed from midnight if the morning smear showed spermatozoa. At 8½ days of pregnancy the rats were injected subcutaneously with 1 ml. of 1 per cent commercial typan blue (Grübler) or solutions of fractions derived from it (*vide supra*) and killed at 20½ days. The number of fœtuses and resorption sites was noted. Fœtuses were removed, fixed in formalin and examined for external malformations. A series of control animals remained uninjected.

Table 2 shows the results obtained.

The demonstration that recrystallized trypan blue gave a resorption-rate (12·5 per cent) that was not significantly different ($0{\cdot}20 > P > 0{\cdot}10$) from the control group (6·7 per cent) suggested that it was the impurities in the crude samples of the dye which were responsible for its high resorption-rate (30·7 per cent). This appeared to be borne out when it was shown that the mother liquor containing these impurities caused resorption (31·0 per cent) at the same rate ($0{\cdot}95 > P > 0{\cdot}90$) as the crude material.

Since the control animals were uninjected, injection trauma was considered as a factor responsible for the resorptions in the experimental series. This consideration could be ruled out, since the resorption-rates given by the series injected with recrystallized trypan blue and the controls do not differ significantly ($0{\cdot}20 > P > 0{\cdot}10$).

Since the main impurity in crude trypan blue (Grübler), apart from sodium chloride, was the red chromogenic material, it was considered that this compound might be the causative resorptive agent. Tests with the impurity showed that the resorption-rate (20·9 per cent) although significantly ($P < 0{\cdot}01$) higher than that of the controls fell between that of recrystallized trypan blue and the crude trypan blue (Grübler) (Table 2).

All fractions—including the pure red fraction which contained no trypan blue at all—were found to cause gross abnormalities, but $\chi^2$ tests of significance failed to show differences in abnormality-rates between the experimental groups due to the small number of externally malformed fœtuses. All fœtuses are now being dissected to demonstrate cardio-vascular abnormalities.

Although the activity of the recrystallized trypan blue cannot be considered to have been disproved, it would appear that the red impurity is more active so far as fœtal resorptions are concerned. An attractive explanation of why the mother liquor was more active than the red impurity itself is that the red compound, being diffusible, is rapidly excreted when injected alone and may not be present at an optimal concentration at the correct period of fœtal development. When, as in the mother liquor or crude trypan blue, the red compound and trypan blue are injected together, the excretion of the red compound may be delayed by partial 'blocking' of the reticulo-endothelial system by trypan blue. Another point that should be mentioned is that the red fraction, as eluted from the cellulose column, contained only the main impurity and that other, possibly more active, compounds which were present in the crude trypan blue or the mother liquor may have been left on the column. Finally, it should be pointed out that the amount of total dye injected into the group of rats receiving the red impurity was only a half that received by the other groups.

Recently, a Wistar strain of rat has been subjected to the experimental treatment outlined above using 1 ml. of 1 per cent crude trypan blue (Grübler) (Table 2). Whereas there is no difference in resorption-rates between control Wistar and M.R.C. hooded rats ($P$ about 0·8) the Wistar rats show a significantly higher ($P < 0{\cdot}01$) resorption-rate (56·2 per cent) (and also a very much higher abnormality-

rate among survivors) than do the M.R.C. hooded rats (30·7 per cent). Experiments with the various fractions of crude trypan blue are now being repeated on the more susceptible strain especially with the view of examining the rates of congenital malformation in the survivors.

F. Beck
B. Spencer
J. S. Baxter

Departments of Anatomy and Biochemistry,
University College,
Cardiff.
March 22.

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## Antileukæmic Activity of Glycyrrhetinic Acid

Recently some pharmacologically interesting activities of glycyrrhetinic acid corresponding to those observed with the adrenal cortical hormones have been shown ; for example, a DOCA-like effect on sodium and potassium metabolism in normal people[1] and an anti-inflammatory activity in different animal tests[2].

It seems that the drug acts through the adrenals, because no effect on the sodium and potassium balance in adrenalectomized rats has been established.

Investigations to obtain a substance like adrenal cortical hormone by transforming the carboxyl-group of glycyrrhetinic acid in a ketol group and the 3-OH group in an oxo-group did not enhance or change the activity[4].

Experiments carried out recently by L. M. Atherden[5] have contributed to the explanation of the mode of action of glycyrrhetinic acid. This author showed that a direct effect on the steroid enzyme system inhibits the metabolism of progesterone and 11-deoxycorticosterone. In this test 11-deoxoglycyrrhetinic acid inhibits to a small extent only. This demonstrates that the 11-oxo-group is required for inhibition.

The purpose of this communication is to describe a further parallel between the activity of adrenal cortical hormones and glycyrrhetinic acid.

E. R. Heilman and E. C. Kendall[6] have shown that cortisone retards growth of malignant lymphosarcoma in mice. Similar results have been obtained by J. B. Murphy and E. Sturm[7] with adrenal cortical hormones in oil : in rats, development of the disease after inoculation with leukæmic cells has been prevented. About 50 per cent of the rats survived while the survival among controls was less than 5 per cent.

Clinical investigations have also demonstrated the effectiveness of cortisone therapy in acute leukæmia, especially in the lymphoblastic variety of acute leukæmia[8].

We have found that glycyrrhetinic acid has a similar activity on the development of transplantable Oberling–Guérin myeloma in rats : α- and β-glycyrrhetinic acid react qualitatively in the same way (Table 1, *b* and *c*) ; but it seems that the α-compound is more active. Also the corresponding 3-oxo-compounds (*d* and *e*) behave analogously. With the 3-oxo-20-ketolacetate (*f*) of glycyrrhetinic acid we have obtained different results with different kinds of leukæmias : with the Oberling–Guérin myeloma some activity has been noted, whereas a lymphatic tumour of the mouse (leukæmia 1210) does not seem to be sensitive to the drug. The compound 3-oxo-

Table 1. Effect of Glycyrrhetinic Acid and Derivatives on the Development of Transplantable 'Oberling–Guérin' Myeloma in Rats

| Compound | Daily dose (mgm./kgm.* intraperitoneally) | No. of animals | Mortality No. of animals | Average tumour weight treated / Average tumour weight controls | Change in body-weight after 12 days Treated / Controls |
|---|---|---|---|---|---|
| (*a*) Cortisone | 50 | 9 | 0 | 0·45 | + 0 / + 20 |
| (*b*) 18 *a*-Glycyrrhetinic acid | 80 | 9 | 1 | 0·32 | + 23·1 / + 25·4 |
| (*c*) 18 *a*-Glycyrrhetinic acid | 80 | 25 | 0 | 0·47 | + 15·5 / + 19·8 |
| (*d*) 3-Oxo-18 α-glycyrrhetinic acid | 80 | 20 | 2 | 0·33 | + 20·2 / + 18·3 |
| (*e*) 3-Oxo-18 β-glycyrrhetinic acid | 80 | 17 | 2 | 0·46 | + 12·8 / + 17·5 |
| (*f*) 3-Oxo-20-ketolacetate-18 β-glycyrrhetinic acid | 50 | 5 | 0 | 0·42 | + 17·0 / + 13·8 |
| (*g*) 11-Deoxo-18 β-glycyrrhetinic acid | 100 | 10 | 0 | 0·88 | + 17·7 / + 14·6 |
| (*h*) 3-Oxo-18 α-glycyrrhetinic-β-dimethylaminoethylester methansulphonic salt | 50 | 10 | 1 | 0·91 | + 19·0 / + 24·5 |
| (*i*) 3-Oxo-18 β-glycyrrhetinic-β-dimethylaminoethylester methansulphonic salt | 50 | 10 | 4 | 0·94 | + 8·2 / + 13·3 |
| (*k*) 3-Acid-hydrogen-succinate of α-glycyrrhetinic disodic salt | 50 | 10 | 1 | 0·65 | + 14·40 / + 23·17 |
| (*l*) 3-Acid-hydrogen-succinate of β-glycyrrhetinic disodic salt | 50 | 10 | 2 | 0·70 | + 21·20 / + 23·17 |

* Duration of treatment : 11 days starting 24 hr. after tumour implantation.

18-α-glycyrrhetinic acid (*d*) was also active against leukæmia 1210.

A negative result was obtained with 11-deoxo-glycyrrhetinic acid (*g*). In order also to carry out experiments with water-soluble compounds, which eventually could show a higher activity, we have tested the β-dimethylaminoethylester of 3-oxo-β-glycyrrhetinic acid (*i*), but it was inactive. Since the β-acid in comparison with the α-acid is much more difficult to hydrolyse[8], we have investigated also the corresponding α-acid ester (*h*): it has proved to be inactive as well. The β- and α-acid hydrogen succinates of glycyrrhetinic acid are also inactive. These 3-succinates have a high anti-inflammatory activity (R. S. H. Finney and G. F. Somers[2]).

It is of interest to note that the glycyrrhetinic compounds do not inhibit the increase in body-weight as in the case of cortisone (Table 1). We have not yet been able to establish whether this fact is in part due to retention of water.

With other tumours, for example, mouse sarcoma 180, no activity was observed with any of the listed compounds. Tumour growth was determined by weighing the localized excised tunours 12 days after subcutaneous implantation of a rat myeloma cell suspension or 8 days after transplantation of the mouse sarcoma 180. Adult Wistar rats or stock albino mice of both sexes were used throughout the experiments.

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W. Logemann
F. Lauria
Chemical Research Laboratory,
G. Cudkowicz
J. Franceschini
Biological Research Laboratory,
Istituto 'Carlo Erba' per Ricerche Terapeutiche,
Milan.

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## Protective Effect of Cysteine against Retinal Degeneration induced by Iodate and by Iodoacetate

Acute degenerative lesions of the neuro-epithelium and of the pigment layer of the retina can be readily induced in the rabbit by a single intravenous injection of five different agents, namely, sodium iodate[1], sodium iodoacetate[2], dithizone[3], the primary phenoxyalkanes[4] and sodium fluoride[5]. To be effective, sodium iodoacetate and sodium fluoride have to be given in sub-lethal doses, and only some 66 per cent and some 20 per cent, respectively, of animals treated with these agents show ophthalmoscopic lesions. With the remaining substances, well-tolerated doses produce retinal lesions in all rabbits treated. The severity of the lesion produced by an agent varies from animal to animal, so that it is difficult to be sure whether there is a fundamental difference between the reactions produced by these five agents; however, dithizone probably gives the most severe and rapidly developing lesion, while sodium fluoride gives the most localized reaction ophthalmoscopically and histologically. Ophthalmoscopic lesions are occasionally seen within a matter of a few hours, but more often not until about 48 hr.; fully established lesions are present almost always within 5 days.

These five agents do not seem to have very much in common chemically, nor apparently biologically; iodoacetate is a versatile enzyme inhibitor, dithizone produces experimental diabetes, and sodium fluoride is known mainly as an inhibitor of glycolysis. The mode of action of these five agents in producing retinotoxic damage is therefore obscure, and failure to obtain a protective effect from the use of possible protective substances—ascorbic acid[6] and vitamin E[7]—has not helped to clarify matters. In the rat, malic acid is protective against the lethal effect of iodoacetate, but enhances the retinal damage[8].

In the course of some work on possible protective substances, there was some suggestion that methionine might prove protective against the retinotoxic effect. The results were, however, not convincing, and as methionine is regarded as breaking up in the body into cystine and choline, and these two substances are antagonistic in experimental liver damage, it was thought best to study them separately. A preliminary trial with choline and cystine in the fasted rabbit suggested that cystine 50 mgm. intravenously 90 min. before and 90 min. after the administration of sodium iodate 17·5 mgm./kgm. intravenously was protective in 3 out of 4 animals treated. In contrast, choline in doses of 30 mgm. similarly administered was ineffective. Further work was continued with cysteine instead of cystine as it is more soluble and larger doses could be readily given intravenously. The procedure employed was to administer intravenously L (+) cysteine hydrochloride 300 mgm./kgm. (using 1 ml. of water per 100 mgm. of cysteine) on 5 days: twice on the day of the injection of the retinotoxic agent (90 min. before and 90 min after the injection) and once daily on the two previous days and the two subsequent days. The results obtained are shown in Table 1.

Table 1. Effect of Repeated Doses of Cysteine Intravenously in Rabbits treated with Five Different Retinotoxic Agents

| Retinotoxic agent | Dose (mgm./kgm.) | No. of animals treated | No. died | Retinal lesions: Expected* | Present | Absent |
|---|---|---|---|---|---|---|
| Sodium iodate | 25 | 10 | 0 | 10 | 0 | 10 |
| Sodium iodoacetate | 25 | 13 | 2 | 5–6 | 1† | 10 |
| „ „ | 30 | 16 | 8 | 5 | 5† | 3 |
| „ „ | 35 | 5 | 4 | 1 | 0 | 1 |
| Dithizone | 45 | 6 | 1 | 5 | 5 | 0 |
| 1 : 5 Di(*p*-amino-phenoxy)-pentane | 5 | 10 | 1 | 9 | 9 | 0 |
| Sodium fluoride | 50 | 12 | 3 | 2 | 2 | 7 |

* In the case of sodium iodoacetate some 50 per cent are expected to show ophthalmoscopic lesions with doses of 25 mgm./kgm., 66 per cent with doses of 30 mgm./kgm., and almost all survivors with doses of 35 mgm./kgm. With sodium fluoride 50 mgm./kgm. some 20 per cent of survivors should show lesions. With the other agents 100 per cent of treated animals should be affected with the dosage employed.
† These lesions were exceptionally mild both ophthalmoscopically and histologically compared to controls not treated with cysteine.

It will be seen that cysteine is fully protective against the retinotoxic effect of sodium iodate; the protective effect is only slightly less with sodium iodoacetate, judged by the fact that even with the larger doses of iodoacetate the ophthalmoscopic and histological reactions were very much milder in cysteine-treated animals than in controls. It would seem that cysteine has no protective effect against the remaining three retinotoxic agents listed.

These results suggest that the five retinotoxic agents known do not all exert their effect through the same mechanism, or that fuller doses of cysteine, and more effective methods of administration, might give control of the three agents uncontrolled in the present observation. Bearing on this latter possibility is the fact that protection appeared to be less effective when cysteine was given less intensively (either intravenously on the day of the injection of iodate only, or in the drinking water for several days).

ARNOLD SORSBY

Wernher Research Unit on Ophthalmological Genetics (Medical Research Council), Royal College of Surgeons, W.C.2.

RONALD HARDING

Royal Eye Hospital, S.E.1.

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## Influence of 5-Hydroxytryptamine on Renal Function in Extracorporeal Circulation

A REDUCED renal blood flow and glomerular filtration-rate, despite a mean aortic pressure over the filtration-level, is a feature inherent to cardio-pulmonary by-pass operations. Yet the maintenance of an adequate renal circulation is of primary importance in preventing ischæmic damage to the kidneys[1], and much research has been directed toward this special problem in the use of heart-lung machines. Accurate measurements on the critical flow-rate in various forms of by-pass were recently reported by Morris *et al.*[2].

Another harmful event in the use of current heart-lung machines is the destruction of thrombocytes. This seems to happen without regard to the type of oxygenator used[3] and can reach considerable dimensions. Recently my colleagues and I reported our observations on the humoral blood trauma in extra-corporeal circulation[4]. The destruction of platelets was followed by a release of 5-hydroxytryptamine into the blood stream, resulting in elevated levels of this substance in the plasma and in its appearance in the urine. The changes in the urinary output, not earlier reported, had the same trend as during the use of heart-lung machines. Table 1 shows the actual values and the amount of 5-hydroxyindoleacetic acid, the main excretory product of 5-hydroxytryptamine, in the urine in periods before and during the extra-corporeal circulation. The urinary output decreased in three dogs of four, from an average of 0·15 ml./min. before extracorporeal circulation to 0·07 ml./min. during this circulation. In one dog the reverse was observed. Despite the elevated levels of 5-hydroxy-tryptamine in the plasma (on the average from 0·025 μgm./ml. to 0·045 μgm./ml.), the 5-hydroxy-indoleacetic acid excretion measured per time unit showed a decreasing trend. This can scarcely be explained on any basis other than a renal factor. There is evidence that the renal excretion of 5-hydroxyindoleacetic acid parallels that of para-amino-hippuric acid[5,6]. If this theory is correct, the 5-hydroxyindoleacetic acid excretion is dependent on both glomerular filtration and tubular excretion, and the changes in renal perfusion conditions in extra-corporeal circulation explain as such the change under consideration. This concept is confirmed by the observation of a low urinary content of 5-hydroxy-indoleacetic acid in the oliguric phase after open cardiac surgery[7] and it indicates that the urinary excretion of 5-hydroxyindoleacetic acid can give an unreliable impression of the decomposition of 5-hydroxytryptamine in conditions with a renal suppression. The possible role of renal pathology is discussed elsewhere[8].

Table 1. URINARY OUTPUT AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) EXCRETION IN EXTRACORPOREAL CIRCULATION

| | Before extracorporeal circulation | | During extracorporeal circulation | |
|---|---|---|---|---|
| | Urine flow | 5-HIAA | Urine flow | 5-HIAA |
| Dog 1 | 0·14 ml./min. | 1·0 μgm./min.<br>7·1 μgm./ml. | 0·09 ml./min. | 0·4 μgm./min.<br>4·0 μgm./ml. |
| Dog 2 | 0·23 ml./min. | 0·7 μgm./min.<br>3·1 μgm./ml. | 0·07 ml./min. | 0·4 μgm./min.<br>5·0 μgm./ml. |
| Dog 3 | 0·09 ml./min. | 0·6 μgm./min.<br>6·5 μgm./ml. | 0·04 ml./min. | 0·4 μgm./min.<br>9·0 μgm./ml. |
| Dog 4 | 0·11 ml./min. | 0·8 μgm./min.<br>7·3 μgm./ml. | 0·28 ml./min. | 0·3 μgm./min.<br>0·9 μgm./ml. |

Considering together the reduced urinary flow and the release of 5-hydroxytryptamine from disintegrated platelets, it seems reasonable that these two phenomena are to some degree interrelated, especially in view of the antidiuretic properties of 5-hydroxytryptamine. There is no general agreement with the original concept of Erspamer[9] that the physiological role of 5-hydroxytryptamine is that of a renal hormone. There are, however, several studies, well controlled to avoid the interference of hypotension, that show a clear-cut antidiuretic effect of exogenous 5-hydroxytryptamine[10,11]. According to these studies, infusions of 5-hydroxytryptamine at dose-levels of 5–15 μgm./kgm./min. had a definite antidiuretic effect in dogs and rats. Also, 5-hydroxy-tryptamine infused into normal people reduced the renal blood flow and glomerular filtration-rate[12]. The same antidiuretic effect certainly applies to endogenously released 5-hydroxytryptamine. Of special interest in this connexion are the findings of Watkins[13]. He observed that the renal suppression began already within 5 min. after the start of a dog by-pass at an adequate perfusion and arterial pressure and with minimal acid-base change. This type of a rapid renal suppression is rather to be related with some humoral agent in the circulating blood than with perfusion and pressure conditions.

Several factors in the use of heart-lung machines, many of them common in major surgery in general, can affect the renal function, perfusion rates clearly playing a central part. The release of 5-hydroxy-

tryptamine, a substance with antidiuretic properties, in extracorporeal circuits, with a simultaneously reduced urinary output, suggests that this substance may also play a part in the renal hæmodynamics of by-pass operations and major surgery in general. Also the liberation of adenosine triphosphate[4] has to be taken into account since this substance releases antidiuretic hormone from the posterior pituitary[14]. Clinical studies are in progress to clarify further the problem under consideration.

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M. H. Frick

Wihuri Research Institute,
Helsinki.

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## PATHOLOGY

### Anti-Inflammatory Activity of Salicylate

Salicylate uncouples oxidative phosphorylation reactions[1], and the possible relation of this major action on cellular metabolism to other effects of the drugs is of great interest[2]. One of the most important properties of salicylate is its anti-inflammatory actions, both in rheumatic disease and in experimental inflammatory states[3]. Adams and Cobb[4] observed a general parallelism between uncoupling activity and the inhibition of erythema in the guinea pig by a series of non-hormonal anti-rheumatic drugs, including salicylate. They suggested a possible connexion between uncoupling ability and therapeutic activity but found that 2 : 4-dinitrophenol, which is a more powerful uncoupling agent than salicylate, failed to affect the erythema test. We have observed that salicylate, but not 2 : 4-dinitrophenol, also modifies the response to the passive cutaneous anaphylaxis test in the guinea pig.

In this test, the animals, after depilation, were given an intradermal injection of antibodies to pneumococcal type III polysaccharide, followed 24 hr. later by the intravenous injection of the antigen and pontamine blue. A progressive formation of œdema and leakage of the circulating dye then occurs in the areas of skin in which the antibodies have been injected. Animals given an intraperitoneal injection of salicylate (500 mgm./kgm. body-weight), 2 hr. before the injection of the antigen and dye, showed a reduced leakage of the circulating dye, but the area in which the extravasation of dye occurred was not changed. Similar treatment with 2 : 4-dinitrophenol (20 mgm./kgm. body-weight) had no effect compared to corresponding control animals.

These observations suggest that the anti-inflammatory action of salicylate, as exerted in the erythema and passive cutaneous anaphylaxis tests, does not result from an uncoupling action on oxidative phosphorylation processes and must be produced by another mechanism.

Full details of the experiments described above will be published elsewhere.

V. Marks
M. J. H. Smith

Chemical Pathology Department,
King's College Hospital Medical School,
Denmark Hill,
London, S.E.5.

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### Fate of Homologous Adult Spleen Cells injected into New-born Mice

During a series of experiments involving radiation chimæras, it became increasingly evident that it would be necessary to consider the results in terms of immunological tolerance of the actively acquired type. At the time a cytological marker was being used for tracing donor cells in the radiation chimæras, following the technique of Ford, Hamerton, Barnes and Loutit[1]. It was decided to use the same marker in tracing cells injected into new-born animals in order to determine, by a direct method, to what extent chimærism is a necessary adjunct to tolerance. There is considerable circumstantial evidence that this is indeed the case[2,3].

$5 \times 10^6$ spleen cells of the *T6/T6* strain were injected intravenously into either *C*⁻ or *CBA* litters within 24 hr. of birth. In three cases *C*⁻ cells were injected into *T6* babies. At various time-intervals after injection, animals were killed and the spleens were prepared for cytological analysis by a modification of the technique of Ford and Hamerton[4]. Table 1 records the results of preliminary experiments. Injection of homologous adult spleen cells into the new-born animals resulted in splenomegaly and runting, a well-known phenomenon[2,5,6].

The rates of division in all spleens investigated, except that of the six-months-old animal, were abnormally high and there were other signs of an

Table 1. A Cytological Analysis of the Spleens of Animals Injected at Birth with Adult Spleen Cells, Chromosomally Marked

| Donor | Recipient | Age of recipient in days, at death | No. of donor cells found | No. of host cells found |
|---|---|---|---|---|
| *T6* | *CBA* | 4 | 0 | 22 |
| *T6* | *CBA* | 4 | 0 | 20 |
| *T6* | *CBA* | 6 | 0 | 73 |
| *T6* | *CBA* | 6 | 0 | 57 |
| *T6** | *C*⁻ | 8 | 3 | 120 |
| *T6* | *CBA* | 10 | 0 | 367 |
| *T6* | *CBA* | 10 | 0 | 20 |
| *T6* | *CBA*† | 180 | 0 | 20 |
| *C*⁻ | *T6* | 3 | 4 | 34 |
| *C*⁻ | *T6* | 6 | 0 | 54 |
| *C*⁻ | *T6* | 21 | 0 | 74 |

* Hyperimmune to *C*⁻
† Bearing a *T6* skin graft.

immunological activity which is thought to be basically a graft-versus-host attack. The only thing lacking in the spleens analysed (Table 1) was the high proportion of dividing donor type cells which has been postulated[2]. Contrary to expectation, splenomegaly in young mice injected at birth with homologous adult spleen cells does not appear to be due primarily to proliferation of the donor cells. Recently, Isacson[7] came to the same conclusion on rather less-direct evidence. Biggs and Payne[8] reported that enlarged spleens in nineteen-day-old chick embryos injected with homologous spleen cells at 14 days of incubation contained roughly equal proportions of donor and host cells.

A more comprehensive series of experiments will be published when the results have been fully analysed.

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A. J. S. Davies
Sheila M. A. Doak

Chester Beatty Research Institute,
Fulham Road,
London, S.W.3.

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## Proliferative Capacity of Acute Leukæmia Cells

Several investigations have been carried out with the purpose of detecting some peculiarities in the deoxyribonucleic acid of leukæmic cells, particularly its content, its base composition and several of its physico-chemical characteristics. It can be expected that more valuable information might be obtained from the study of deoxyribonucleic acid metabolism than from the simple analysis of its static properties. The incorporation of several precursors of deoxyribonucleic acid in the total leucocyte pool has been investigated with chemical methods[1–3]. An increased uptake has been detected in leukæmic marrows as compared with normal ones[1,2]. However, such a study does not allow a direct comparison between normal and pathological marrows since with these techniques the importance of different leucocyte populations of various degree of maturation is necessarily overlooked. That holds particularly for acute leukæmia cells, of which the normal counterparts (that is, normal myeloblasts) represent only about 4 per cent of the whole cellular population in a normal bone marrow.

Radioautography is considered at present as the best technique for an investigation at a cellular level. With this technique Lajtha[4] has studied the incorporation of phosphorus-32 and of adenine-$^{14}$C into the deoxyribonucleic acid of human bone marrow cells *in vitro* and obtained valuable results concerning the kinetics of cellular division.

In the past few years the availability of thymidine labelled with tritium has allowed the use of the most specific precursor of deoxyribonucleic acid (thymidine) and, at the same time, it has enormously increased the resolution of the technique (tritium labelling)[5–8].

The present investigation deals with the incorporation of tritium-labelled thymidine, as studied with a radioautographic technique in bone marrow cells of five normal individuals and of 13 patients with acute leukæmia.

Bone marrow was obtained by sternal puncture and aspiration into a heparinized syringe, diluted 1/1 with Eagle medium and transferred into siliconized test tubes. $^3$H-thymidine (specific activity 890 mc./m*M*) was added to a final concentration of 2·5 μc./ml. Incubation was carried out in a rotating system at 37°. After 1, 3 and 5 hr. smears were made on gelatine-coated slides. The smears were fixed in Carnoy's solution and, after several washings in distilled water, radioautographic preparations were obtained according to the current technique[8]. The percentage of labelled cells was determined, in each case, counting at least 100 myeloblasts for the normal and 2,000 blast cells for the leukæmic marrows.

The strikingly lower incorporation of thymidine labelled with tritium in acute leukæmia cells as compared to that detected in normal myeloblasts is shown in Table 1. No significant variations in the percentage of labelled cells were observed among the different normal bone marrows. In all cases, between the first and the fifth hour of incubation, the percentage of labelling did not significantly increase.

Table 1. Percentage of Labelled Myeloblasts after 1 hr. Incubation

| Normal bone marrows | Acute leukæmias | | | |
|---|---|---|---|---|
| | Stem cells | Myeloblastic | Monoblastic | Lymphoblastic |
| 50<br>45<br>35<br>40<br>35 | 1·0<br>1·4<br>3·3<br>2·7<br>9·1<br>2·8<br>1·0 | 0·2<br>9·4<br>4·0<br>7·0 | 5·4 | 1·5 |
| Mean value 41 ± 6·5 | 3·0 ± 2·8 | 5·1 ± 4 | | |

It is well known that the incorporation of thymidine, as evidence of synthesis of deoxyribonucleic acid, occurs only as a preparation for later mitosis. It is therefore considered to be the most reliable index of the proliferative capacity of a tissue. The low percentage of labelling detected in acute leukæmia cells therefore indicates an extremely low proliferative capacity of these elements. Our results are in good agreement with those previously suggested by other investigators utilizing bone marrow cultures and the stathmokinetic test[10,11].

These results are in contrast with the current opinion of a high mitotic activity of leukæmic tissues, and suggest that in acute leukæmia elements, as compared to their normal counterparts, another important functional defect is present associated with the well-known incapacity to differentiate. This consideration might be of some importance in regard to a more rational therapeutic approach in acute leukæmia.

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Felice Gavosto
Giovanni Maraini
Alessandro Pileri

General Medical Clinic,
Turin Medical School.

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## RADIOBIOLOGY

### Removal of Internally Deposited Americium by Chelating Agents

During recent years there has been considerable interest in the use of chelating agents to remove certain radioelements deposited in the body. Ethylenediamine tetraacetic acid has been investigated extensively, and more recently there have been reports that diethylenetriamine pentaacetic acid is more effective than ethylenediamine tetraacetic acid for the removal of deposited plutonium[1], and that it also increases the excretion of thorium from the body[2]. We have studied the effect of these two chelating agents on the removal of americium-241 from the rat. Preliminary experiments showed that both reagents significantly increased the excretion of this nuclide and that diethylenetriamine pentaacetic acid was approximately twice as effective as ethylenediamine tetraacetic acid.

Groups of three young male hybrid rats of the $F_1$ generation bred from Marshall and August parents were injected intravenously with 0·2 μc. americium-241 as the citrate complex. Treatment with 0·3 m.mole diethylenetriamine pentaacetic acid and 0·2 m.mole calcium gluconate, given by intraperitoneal injection in 2 ml. of solution, was started at times varying from 1 hr. to 7 days after the injection of americium-241 and repeated at weekly intervals. The rats were killed 21 days after the injection of americium-241 and the alpha activities of the liver, femur, carcass and excreta were measured by the technique described by Turner, Radley and Mayneord[3].

In Fig. 1 the distribution of americium-241 twenty-one days after administration to a normal animal is compared with that in animals treated with diethylenetriamine pentaacetic acid at various intervals. The untreated animals contain approximately 40 per cent of the injected activity in the skeleton, 25 per cent in the soft tissues, and excrete about 35 per cent. When treatments with diethylenetriamine pentaacetic acid were commenced at 1 hr., 6 hr. and 7 days after the administration of americium-241, the levels in the skeleton were 7, 10 and 20 per cent, respectively, and the amounts in the soft tissues were reduced to less than 1 per cent of the injected activity. Most of the americium-241 that was removed was excreted in the urine.

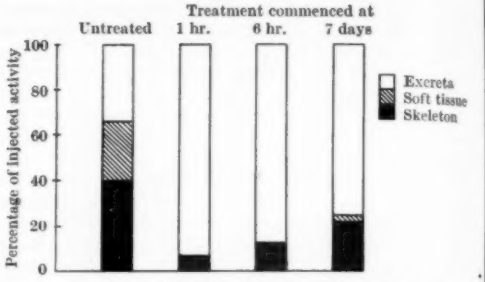


Fig. 1. Effect of diethylenetriamine pentaacetic acid on the distribution and excretion of americium in rats

It is concluded that diethylenetriamine pentaacetic acid can remove americium-241 from the skeleton and soft tissues, and that an appreciable reduction in the body burden can be achieved, particularly when the agent is administered within a few hours after exposure.

This work will be more fully reported in a later publication, which will include a comparison of the effects of multiple and single doses of chelating agent, and the effects of oral and intraperitoneal administration.

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F. D. Sowby*
D. M. Taylor

Department of Physics,
Institute of Cancer Research,
Royal Cancer Hospital,
London, S.W.3.

* Permanent address: Radiation Protection Division, Department of National Health and Welfare, Ottawa, Canada.

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### Radiosensitivity of Mouse Kidney undergoing Compensatory Hypertrophy

We have been interested in assessing the degree or extent of acute radiation damage to the kidneys, particularly as it may relate to late radiation effects on this organ, namely nephrosclerosis[1,2]. Significant acute changes in renal morphology (that is, during the first month), following whole-body irradiation in the lethal dose-range, have not in general been observed[3,4]. In this connexion, it is well known that the kidney of the normal adult exhibits only minimal mitotic activity. Therefore it was thought of interest to ascertain whether the kidney becomes radiosensitive when mitotic activity is stimulated by unilateral nephrectomy. Mitotic activity of the kidney following unilateral nephrectomy has been shown by Rollason[5] and by Sulkin[6] to be largely restricted to the period 48–72 hr. post-surgery.

Female mice of the $(C57L \times A)F_1$ hybrid strain were employed. A total of eighty 10-week-old animals were subjected to left unilateral nephrectomy under ethyl ether anæsthesia. The left adrenal gland was dissected free from the kidney with its blood supply intact, and spared. The excised kidney was gently

blotted and weighed in a glass-stoppered weighing bottle to the nearest milligram. In a series of normal mice, comparison of the left and right kidney weights in each animal showed no more than 5 per cent difference—the right kidney being the larger in every case. These mice were divided into four sub-groups, approximately 15 each. One sub-group was X-irradiated in a single whole-body exposure to 690 r. (a high sublethal dose) 3 hr. after surgery. Another sub-group constituted a non-irradiated control group. A third sub-group of nephrectomized mice was X-irradiated similarly (690 r.) 48 hr. after surgery, with the fourth sub-group serving as the non-irradiated controls. A fifth sub-group was irradiated (690 r.) 1 hr. prior to unilateral nephrectomy. Of the 80 original mice, 75 were killed three weeks after surgery (a time at which compensatory hypertrophy of rat kidney has been shown to reach a plateau[5]), and the remaining right kidney removed and weighed. Kidneys from four of the original mice were removed 48 hr. after nephrectomy, and fixed in formalin for histological examination. One mouse of the 80 died 7 days post-irradiation. For determination of mitotic activity, kidney sections were stained with Feulgen reagent, and mitoses per 50 high-power fields were calculated. The X-radiation source was a Westinghouse therapy unit, operated at 250 kVp. The radiation factors were: 15 m.amp.; filter 0·5 mm. copper plus 1 mm. aluminium; half-value layer 1·5 mm. copper; target-skin distance 100 cm.; dose-rate 30 r. per min., in air.

A summary of the results showing the percentage increase in kidney weight in each animal, relative to the initial left kidney weight, is presented in Fig. 1. It is evident that in the group irradiated (690 r.) 48 hr. post-nephrectomy, compensatory hypertrophy of the right kidney was not inhibited, as compared with that of non-irradiated, nephrectomized controls. Many of the kidneys in both these groups showed increases in weight up to 50 per cent. By contrast, unilaterally nephrectomized mice exposed to 690 r. 3 hr. post-surgery showed only a minimal increase in weight of kidney—only one kidney showing increase in weight of as much as 21 per cent, the bulk of the remainder showing weight increases of 10 per cent or less.

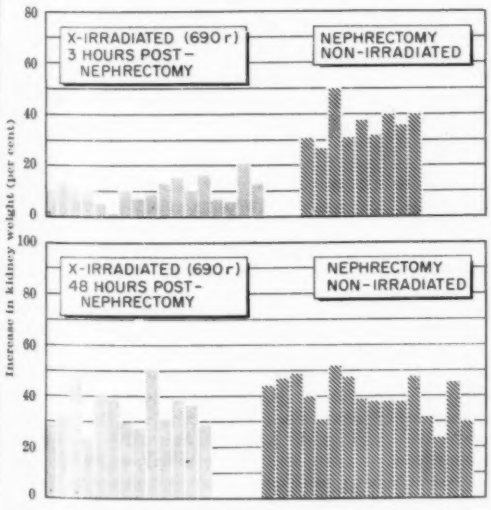


Fig. 1. Inhibition of compensatory hypertrophy of mouse kidney by X-irradiation (690 r.) 3 hr. post-nephrectomy

The weights of the normal left kidneys ranged from 86 mgm. to 159 mgm., with a mean of 121 mgm. The corresponding weights of the right kidneys extirpated from the non-irradiated mice 3 weeks post-surgery ranged from 127 mgm. to 203 mgm., with a mean of 167 mgm. By contrast, weights of the right kidney of mice irradiated 3 hr. post-surgery ranged from 114 mgm. to 170 mgm. (mean of 137 mgm.), as compared to weights of the right kidney of mice irradiated 48 hr. post-surgery with a range of 120–205 mgm. (a mean of 167 mgm.).

Mitotic counts were determined on the right kidneys removed from two non-irradiated control mice 48 hr. after left unilateral nephrectomy, and also on corresponding kidneys from two 690 r. irradiated mice (3 hr. post-nephrectomy), killed 48 hr. after surgery. In the non-irradiated mice, 136 and 147 mitoses, respectively, per 50 high-power fields were observed. By comparison, the kidneys of the irradiated mice (irradiated 3 hr. post-nephrectomy) showed only 7 and 5 mitoses per 50 high-power fields 48 hr. after surgery. (One high-power field contains approximately 400 cells.) The mitotic figures were distributed proportionately between cortex and medulla. Corresponding values for normal, non-irradiated kidneys were 5 and 8 mitoses per 50 high-power fields, respectively.

When 690 r. X-radiation was delivered 1 hr. prior to nephrectomy, no inhibition of capacity of the kidney to undergo compensatory hypertrophy could be detected. The weights of the left kidney from this group (that is, at the time of unilateral nephrectomy) ranged from 98 mgm. to 140 mgm., with a mean of 118 mgm. The weights of the right kidney, that is, three weeks post-nephrectomy, ranged from 129 mgm. to 205 mgm., with a mean of 168 mgm. The percentage weight increase of right kidney over left kidney varied from 30 to 54 per cent, with a mean increase of 42 per cent.

The foregoing results indicate that the mouse kidney is radiosensitive during a critical time period following a stimulus to cell division, namely unilateral nephrectomy. If the irradiation (690 r.) is carried out 2–3 hr. post-nephrectomy, there is a marked inhibition both of mitotic activity at 48 hr., and of compensatory hypertrophy, as measured at 3 weeks post-surgery. By contrast, when irradiation (690 r.) is carried out 48 hr. post-surgery, the capacity of the kidney to undergo compensatory hypertrophy is not impaired. It seems evident that the radiosensitivity of the kidney 3 hr. post-unilateral nephrectomy is somehow related to its cellular activity following upon the stimulus which leads to cell division—presumably preparation for deoxyribonucleic acid synthesis, since Becker and Ogawa[7] have demonstrated an increase in the amount of deoxyribonucleic acid per nucleus under these conditions of mitotic activity in the kidney. Recent studies by Beltz and Applegate[8] on regenerating liver would appear to shed further light on this problem, at the biochemical level. These workers subjected rats to partial hepatectomy, exposed them to 1,500 r. at 12–13½ hr. post-surgery, and killed them 25 hr. after surgery; assay for incorporation of thymidine labelled with tritium into deoxyribonucleic acid by the liver supernatant fractions *in vitro* was then carried out[8]. It was found that the liver supernatants from the irradiated partially hepatectomized rats possessed only 10 per

cent of the capacity of the same tissue fractions from non-irradiated controls to incorporate labelled thymidine into deoxyribonucleic acid. They conclude that the effect of irradiation "is to prevent the synthesis, or the production of zymogen activation of one or more enzymes in the group of enzymes functioning to incorporate thymidine into deoxyribonucleic acid". It is tempting to speculate on the radiation-inhibition of compensatory hypertrophy in the kidney, presented in this report, in terms of similar mechanisms.

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V. J. Rosen, jun.
L. J. Cole

Biological and Medical Sciences Division,
U.S. Naval Radiological Defense Laboratory,
San Francisco 24, California.

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## Uptake of Thymidine and Synthesis of Deoxyribonucleic Acid in Mouse Ascites Cells

Recent studies of the synthesis of deoxyribonucleic acid have suggested that the radiation-sensitive period may occur at a stage in the cell-cycle before the polymerization of new deoxyribonucleic acid begins, that is, during the G1 period[1–3]. It therefore appeared to be of interest to study, in more detail, the assimilation of the precursors into cells and their subsequent incorporation into deoxyribonucleic acid. With this object we have examined the uptake of thymidine labelled with tritium into mouse Ehrlich and Landschutz ascites cells *in vitro*, measuring the total activity present in the cell and the activity incorporated into deoxyribonucleic acid, as a function of time. The distribution of the precursor among the cells of the population was determined by autoradiography. 2 ml. aliquots of suspension containing about $10^8$ cells were incubated at 37° in a medium containing 50 per cent ascitic fluid and 50 per cent fortified Hanks' medium[4] (5 gm. glucose/l.). At the end of the incubation the cells were washed five times with ice-cold Hanks' medium and finally suspended in 4 ml. of the medium. Autoradiographs were prepared in the usual manner, the cells being air dried, fixed with 2 per cent acetic acid or with 45 per cent acetic acid in alcohol and washed before the application of the stripping film. Deoxyribonucleic acid was prepared from 2 ml. of the cell suspension using the detergent method of Kay, Simmons and Dounce[5], the final product being dissolved in 2 ml. of water. The solutions of deoxyribonucleic acid and the cell suspensions were homogenized by treatment with ultrasonics before plating on aluminium planchettes. Determinations of tritium activity were made using a windowless flow counter working in the proportional region with an efficiency of about 15 per cent. Deoxyribonucleic acid contents of the cell suspensions and acid solutions were determined using the modified diphenylamine method of Burton[6].

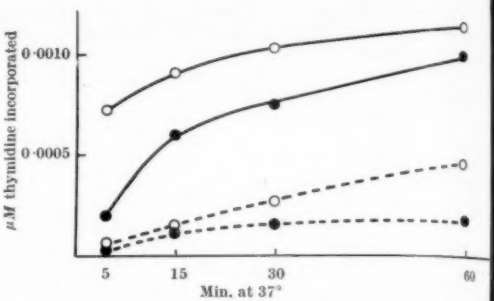


Fig. 1. Incorporation of tritium-labelled thymidine into cells and deoxyribonucleic acid. *a*, 0·001 μ*M*, 2 μc. $^3$H-thymidine. ●—● cells, ●- - -● deoxyribonucleic acid; *b*, 0·01 μ*M*, 5 μc. $^3$H-thymidine, ○——○ cells, ○- - -○ deoxyribonucleic acid

The general pattern of the results obtained for both types of cells is illustrated in Fig. 1. Thymidine is rapidly taken up by the cells, but the rate of incorporation into deoxyribonucleic acid is much slower. The amount of thymidine which the cells can take up is limited. For example, when 0·001 μ*M* of thymidine is added to a suspension containing about $10^8$ cells, nearly all of it is taken up by the cells in 1 hr., but when ten times this amount of thymidine is added the amount taken up in 1 hr. is only slightly increased. The amounts of tritium-labelled thymidine in the cells and in the deoxyribonucleic acid are not increased appreciably by longer periods of incubation (up to 3 hr.). Addition of the other three nucleosides does not influence the amounts taken up. The failure of the cells after 1 hr. to take up more thymidine or to synthesize more deoxyribonucleic acid is not due to a general stopping of the metabolic processes of the cells since the rate of incorporation of orotic acid into the ribonucleic acid continued at a constant rate for at least 2½ hr. Neither can the effect be attributed to damage caused by radiation from the labelled thymidine present in the cells, since the amount of precursor taken into the cells and incorporated into the deoxyribonucleic acid is the same if 0·01 μ*M* of thymidine is added with an activity of 5 μc. or 0·5 μc. It appears that when the cells are incubated in this medium some changes occur or some metabolite is lacking which prevents them from either accumulating thymidine or synthesizing deoxyribonucleic acid for more than a short time.

We have also attempted to ascertain the nature and distribution of the precursor which is present in the cells but not polymerized into deoxyribonucleic acid. Nuclei were prepared using the method of Allfrey *et al.*[7], and the non-nuclear fraction was found to contain scarcely any activity. The autoradiographs also showed that the precursor was concentrated in the nuclei of the cells. Very little activity was lost from the nuclei on standing in Hanks' medium and no exchange was detected when inactive thymidine was added to the medium. It was, however, found that treatment of the cells or nuclei with ultrasonics and centrifuging for 20 min.

at 100,000*g* left considerable amounts of activity in the supernatant fraction. Chromatography of these supernatant fractions (*iso*butyric acid/ammonia solvent) obtained from cells incubated for 1, 5 and 16 min. showed that the activity of tritium was accounted for as 15 per cent thymidine di- and tri-phosphates (these two compounds were assayed together), 25 per cent thymidine monophosphate and 60 per cent thymidine in all cases. These results suggest that thymidine is taken up by the cells and transferred to the nucleus where an equilibrium is rapidly set up between thymidine, thymidylic acid and the di- and tri-phosphate derivatives. Although these compounds cannot be simply washed out from the cells or nuclei, it appears that they are not firmly bound within the nucleus since they can be liberated by destroying the nuclear structure. It seems probable that some part of the precursors may be even more firmly bound within the nucleus since the activity of the supernatant fractions obtained after ultrasonic treatment, added to the observed activity of the deoxyribonucleic acid, accounts, in some cases, for only 60 per cent of the total activity of the cells.

Table 1. Comparison of the Tritium Activity of Cells and Deoxyribonucleic Acid and the Degree of Labelling observed in Autoradiography Experiments

| Minutes incubated at 37° C. | C.p.m./μgm. deoxyribonucleic acid in the cell suspension | C.p.m./μgm. deoxyribonucleic acid in the isolated deoxyribonucleic acid | Average spots per cell in autoradiograph | Fraction of cells labelled in autoradiograph (per cent) |
|---|---|---|---|---|
| 15 | 115 | 32 | 7·1 | 32 |
| 60 | 119 | 79 | 5·5 | 29 |

It has usually been considered that the activity observed, in the nucleus, by autoradiographic procedures after appropriate washing and fixing techniques can be ascribed to the thymidine incorporated into deoxyribonucleic acid[8]. The results of this work show that this is not necessarily valid. It can be seen, for example, in Table 1, that after 60 min. incubation the average amount of tritium-labelled thymidine assimilated per cell is little different from that observed after 15 min. incubation, though more than twice as much of the thymidine has been incorporated into deoxyribonucleic acid. The data from the autoradiographs suggest that the average activity per cell is roughly the same for the two periods of incubation and clearly bears no relationship to the activity of the deoxyribonucleic acid alone. It would therefore appear that the fixing procedure does not remove a significant part of the labelled thymidine, which is incorporated into the cell but not polymerized into deoxyribonucleic acid. To confirm this conclusion a suspension of washed cells was centrifuged and the cells resuspended in (*a*) 2 per cent acetic acid and (*b*) 45 per cent acetic acid in alcohol. After standing for 5 min. at room temperature the suspensions were centrifuged and the activity of the supernatant fractions assessed. In both cases the total activity of the fixing fluids was less than 10 per cent of the total activity of the cell suspension. Table 1 also shows that only one-third of the cells in the suspension have assimilated labelled thymidine. It would therefore appear that under the conditions used in our experiments, the autoradiographic process detects not only the cells which are actively polymerizing new deoxyribonucleic acid but also those which contain precursors in a form not easily removed by the washing and fixing procedures (cf. Harris)[9]. Other workers have attempted to assess mitotic activity from counts of the number of labelled cells in autoradiographs and have found an apparent index considerably greater than that obtained from experiments using colchicine to inhibit cell division[10]. The results described above could provide an explanation for this anomaly.

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A. R. Crathorn
K. V. Shooter

Chester Beatty Research Institute,
Royal Cancer Hospital,
Fulham Road,
London, S.W.3.

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## Effects of Whole-body Irradiation on the Breeding Plumage of the Weaver finch, *Quelea quelea*

There have been recent unsubstantiated reports that individuals of certain migratory species of birds have arrived in their African wintering grounds in breeding plumage instead of their normal eclipse condition[1]. Again, several migratory waders have been collected in Great Britain in unseasonal plumage. Some have been found to be radioactive; an examination has shown in some of the birds stimulation effects of the neuro-endocrine apparatus abnormal for the season (Harrison, J. M., personal communication). There is no evidence that the two factors are related, but the possibility that the anterior pituitary and sexual cycle, involving plumage changes, have been artificially disturbed by radiation could not be overlooked.

In order to investigate whether such phenomena can be produced by radiation, seasonally dimorphic weaver finches (*Quelea quelea*) were imported from Africa. In this species the male periodically assumes a vivid nuptial plumage, and after reproduction moults into non-breeding plumage which is indistinguishable from that of the permanently sombre female[2]. This cyclical assumption of a brilliant nuptial plumage is, as in certain other African weaver finches, under the direct influence of the adenohypophysis[3] and has been adopted by Witschi as a bio-assay test for gonadotrophins. He and his co-workers showed that

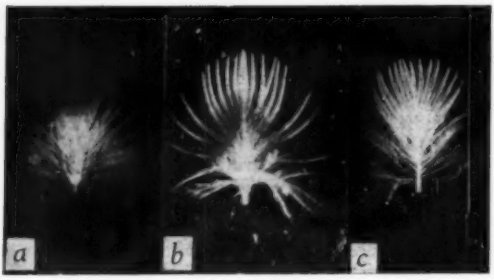


Fig. 1. (*a*) Male feather regenerated after 1,000 r. Tip normal and pigmented but post-irradiation growth is unpigmented. (*b*) Female feather showing band of abnormal melanin deposition after whole-body irradiation of 200 r. (*c*) Unpigmented control female feather

the deposition of melanin in regenerating feathers is a specific reaction to luteinizing hormone[4].

In a preliminary experiment six groups each of 12 individuals (6 males and 6 females) were used. All these were in full breeding condition, all the males displayed the complete nuptial plumage of black facial mask and golden crown and breast, and all the females bright yellow beaks and drab plumage. Feathers were plucked from the black face-mask area of all birds, which were then left for four days, during which new feathers began to grow. Five of the six groups were then subjected to a single whole-body exposure of 15-MeV. X-rays at doses of 50 r., 200 r., 400 r., 800 r. and 1,000 r., respectively. The sixth was retained as a control group. The source of radiation was the 15-MeV. linear accelerator at St. Bartholomew's Hospital[5], and the dose-rate was about 500 r./min.

The feathers that regenerated in the plucked facial mask of male controls were identical with those removed before irradiation. In both cases they were black. In all the irradiated birds except those that received a dose of 1,000 r., the regenerated cock feathers were of the black type and indistinguishable from those of the controls. The regenerated plumage of the three survivors of the 1,000 r. exposure (approximately the *LD*50 for this species with this type of radiation), however, was unpigmented except at the feather-tips, which had been laid down in the four days before irradiation. No melanin was deposited in the parts of the feathers that had grown after irradiation (Fig. 1*a*).

Females of this species do not cyclically assume coloured breeding plumage, and so feathers removed from the facial mask area before irradiation were white and unpigmented. Regenerated feathers in female controls were unchanged, but those in the 50 r., 200 r. and 400 r. groups now displayed a black band of melanin deposition. The tips, which had developed before irradiation, were light and of normal hen colour. After irradiation, however, this normally coloured tip was succeeded by a band of melanin deposition of a kind that normally occurs only in cocks. The feather base was again hen-type and white (Figs. 1*b* and 1*c*). It is important to mention, however, that in the two higher-dosage groups melanin deposition did not occur and the regenerated plumage remained of the normal hen type.

All birds underwent a normal post-nuptial moult into the eclipse condition in July. By September, male birds were once again starting to assume their nuptial plumage.

A second experiment was performed on *Quelea* while the birds were in eclipse condition. Three groups of 24 such specimens were deplumed in the mask area and their feather follicles allowed to reorganize. One group was then given a dose of 100 r. of whole-body irradiation, another 800 r., and the third group was retained as a non-irradiated control. They were then left until the feathers had regenerated, when it was found that in all cases the new feathers were apparently normal, unpigmented and typical of the eclipse condition.

The abnormal deposition of melanin in the plumage displayed by female birds may be indicative of pituitary stimulation induced by the lower irradiations. This, however, appears to be only a temporary condition, for the birds soon reverted to their natural plumage and underwent normal moult cycles. Whether an exposure at a much lower dose-rate would have a prolonged stimulatory effect, and afterwards extend the period of melanin deposition, is not known and is under investigation.

We wish to thank the Nuffield Foundation for its support of this and allied studies.

B. Lofts

Department of Zoology and Comparative Anatomy,
St. Bartholomew's Hospital Medical College,
University of London.

A. J. Marshall

Department of Biology,
Monash University,
Melbourne.

J. Rotblat

Department of Physics,
St. Bartholomew's Hospital Medical College,
University of London.

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## BIOLOGY

### Breeding Sites of Non-Cichlid Fishes in Lake Victoria

Breeding sites in Lake Victoria are known for three of the non-cichlid fishes. The lung-fish, *Protopterus aethiopicus* Heckel, constructs a nest in marginal swamp[1], and the cat-fish, *Clarias mossambicus* Peters[2], and the carp, *Labeo victorianus* Blgr.[3], spawn in temporary streams which flow into the lake after rain.

A study of the food of non-cichlid fishes in the Lake Victoria basin has recently been completed, and some of the findings, when considered in conjunction with other ecological facts, have indicated that in the lake two species, *Bagrus docmac* (Forskal) and *Mormyrus kannume* (Forskal), may breed on exposed rocky shores. The evidence is stated below.

The geological history of Lake Victoria[4] indicates that it has been colonized relatively recently by fishes of swamps and rivers[5]. Several non-cichlid fishes now inhabiting the lake periodically ascend affluent rivers *en masse*, presumably to breed[6]. *Bagrus* and *Mormyrus*, however, do not do this;

Table 1. NON-CICHLID FISHES COLLECTED FROM A ROCKY SHORE IN LAKE VICTORIA

| Species | No. collected | Size-range (cm.) | Median (to nearest cm.) |
|---|---|---|---|
| *Bagrus docmac* (Forskal) | 41 | 3–16 | 8 |
| *Clariallabes petricola* Greenwood | 34 | 3–11 | 6 |
| *Mastacembelus victoriae* Boulenger | 31 | 3–27 | 10 |
| *Clarias mossambicus* Peters | 15 | 3–14 | 11 |
| *Mormyrus kannume* (Forskal) | 8 | 11–20 | 14 |
| *Clarias werneri* Boulenger | 3 | 7–10 | 10 |
| *Engraulicypris argenteus* (Pellegrin) | 1 | 5 | — |
| *Synodontis afro-fischeri* Hilgendorf | 1 | 11 | — |

*Bagrus* takes part in the upstream migrations, but only in relatively small numbers[6], and therefore, like *Mormyrus*, must be assumed to breed within the lake itself. Juveniles of both these species had hitherto only been found in numbers in torrential reaches of the effluent Victoria Nile at Jinja, where they fed on lithophilic insects among the stones of the river bed. In the lake the habitat most resembling this environment is the exposed rocky shore, where many of the same insects (for example, *Afronurus*, *Neoperla*, *Metacnemis* and *Phanostoma*) typically occur. In the Victoria Nile juveniles of both fishes feed on lithophilic insects, but the adults on foods derived elsewhere. In the lake, individuals of these species below 15 cm. are scarcely ever found, but the food of fishes larger than this (of which more than 4,000 have been examined) changes according to their size, so that they feed progressively less on lithophilic organisms as they become older. Thus, although samples of fishes from the lake included no juveniles, they revealed ontogenetic changes in feeding behaviour which paralleled those observed in the river. This may therefore be regarded as additional evidence for supposing that in the lake exposed rocky shores provide the habitat for juveniles.

To test this supposition a small fish-poisoning operation was carried out on February 13 at Entebbe, along a short strip (about 50 m. × 3 m.) of rocky shoreline, exposed to unusually vigorous wave-action. The results (Table 1) confirm expectation in two important respects: they make it virtually certain that in the lake such a habitat provides a breeding site for *Bagrus*; and they emphasize the ecological similarity which exists between rocky shores in the lake and turbulent reaches in associated rivers. Whether or not *Mormyrus* also breeds on such shores must at present remain an open question, although the evidence given earlier indicates that this may be so. It should be noted that one Entebbe specimen of 20 cm. total length was a ripe female.

The presence of numerous *Clariallabes petricola* Greenwood is particularly informative, since not only is this the first record of this species from the lake proper but its type habitat[7] is the very spot where juvenile *Bagrus* and *Mormyrus* were found in the Victoria Nile. One female (6 cm. total length) from Entebbe had ripe gonads. Here it should be mentioned that another fish to be newly recorded from Lake Victoria, and also collected previously from the Victoria Nile site[7], is *Garra johnstoni* (Blgr.), which was found recently on an exposed loose-rock shore at Entebbe by Mr. J. P. C. Greenway.

Another significant feature of the results is the abundance of *Mastacembelus victoriae* Blgr., which was actually the commonest fish on the site. Like *C. petricola*, this species had previously been found to be very common on the bed of the Victoria Nile[8], but had hitherto been encountered only rarely in the lake, presumably because the appropriate habitat there had not been sampled. Thus, these observations correspond well with the report that in Lake Nyasa *Mastacembelus shiranus* Günther was encountered commonly only when fish-poisoning was carried out on rocky shores[9]. At Entebbe three *M. victoriae* (10, 16 and 26 cm. total length) were ripe females.

Although the specimens of *C. mossambicus* are too large to indicate that breeding was occurring in this site, it is perhaps significant that fishes of this estimated age (6–12 months) should be found in such an environment where, as in the temporary streams in which they are spawned[2], there is vigorous water movement.

From the observations recorded here it may be concluded that the fauna of wave-washed rocky shores in Lake Victoria closely resembles that of turbulent reaches in associated rivers (from which it presumably originated), and that consequently the rocky shore may be considered to constitute an important transition habitat able to accommodate the least-adaptable stages in the life-histories of those riverine fishes in process of becoming lacustrine. It follows that systematic studies of rocky shores may be expected to produce much valuable information concerning the ecology and evolution of non-cichlid fishes in the lake.

*B. docmac* is one of the non-cichlids caught most frequently by fishermen in Lake Victoria. That it should breed on rocky shores promises well for its future utilization, for it is likely that in the normal course of events neither man nor natural predators will interfere significantly with such a habitat.

I am grateful to Messrs. D. H. Rhodes and J. P. C. Greenway for assisting with the fish-poisoning operation, and to Mr. P. H. Greenwood for identifying and estimating the age of the Clariidae.

A full account of the work leading to these conclusions will be published elsewhere.

PHILIP S. CORBET*

East African Fisheries Research Organization, Jinja, Uganda.

* Present address: Virus Research Institute, Entebbe, Uganda.

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## Occurrence of Free D-Serine in the Earthworm

LOMBRICINE was first isolated from the earthworm by Thoai and Robin[1]. Analysis of the crystalline material isolated in this laboratory[2] showed that the serine component was of the D-configuration[3]. Serine ethanolamine phosphodiester, which has been shown to be a precursor of lombricine in the earthworm[4], has been isolated in the crystalline form and shown to contain D-serine[5].

We now wish to report the finding of free D-serine in the acid-extractable free amino-acid pool of the earthworm.

The earthworms used were deep-frozen material of mixed species, but predominantly *Allobophora caliginosa* and *Octolasium cyaneum*. Extracts were prepared and fractionated as described[5] for the isolation of lombricine and serine ethanolamine phosphodiester; those fractions containing serine were identified chromatographically, collected and purified by repeated passage through 'Dowex-50' ($NH_4$-form).

The chromatographically pure serine was crystallized from a small volume of hot aqueous ethanol. Two crops were invariably obtained; the first crystallized easily on the addition of 2–3 volumes of ethanol to the aqueous solution, while the second usually required considerably higher ethanol concentrations, or the addition of ether, before crystallization occurred. 3 kgm. of frozen earthworms yielded 45 and 42 mgm. of the first and second crops, respectively. Both samples were shown to be pure serine by carbon, hydrogen and nitrogen analysis and by two-dimensional chromatography in ethanol/formic acid/water (7 : 1 : 2) and water-saturated phenol.

Examination of the optical rotation (in water) of the two samples showed crop 2 to be lævorotatory, while crop 1 was optically inactive.

In order to exclude the possibility of racemization during extraction and subsequent fractionation, a sample of authentic L-serine was dissolved in 1·5 *N* perchloric acid and the entire isolation procedure repeated with this solution. The serine was recovered in good yield and crystallized. It was found to be lævorotatory ($[\alpha]_D^{22\cdot0} = -7\cdot37$ in water, C = 4·2 per cent).

The optical purity of the serine samples was checked using a partially purified D-amino-acid oxidase preparation[6] which also contained a very active catalase. With crop 1 the oxygen uptake in 3 hr. was 88 per cent of that theoretically expected (1·0 μatom/μmole) for DL-serine in the presence of catalase. No oxygen uptake occurred with crop 2 or with the control L-serine re-isolated by the standard procedure. The possibility that the D-serine found in the perchloric acid extract of the earthworms was due to racemization can thus be excluded. In this connexion Minthorn *et al.*[7] have also shown, using the two serine enantiomorphs labelled with carbon-14, that the optical purity of serine was unaffected by techniques similar to those employed in the present experiments. Differential crystallization of DL-serine and one of the optically active enantiomorphs from a mixture has also been reported by these workers[8].

The present results thus establish the occurrence of D-serine in the free amino-acid pool of the earthworm, and, in view of the almost total recovery of the serine contained in the original extract, suggest that about 25 per cent of the free serine present is of the D-configuration. To the best of our knowledge this is the first occasion on which a free D-amino-acid has been unequivocally shown to be present in animal tissue. However, two unconfirmed early reports, on the finding of D-ornithine in fish liver[9], and of DL-alanine and DL-glutamate in goose and insect tissues, respectively[10], are referred to by Neuberger[11].

It would seem likely from the present findings that, in the earthworm, serine ethanolamine phosphodiester, and therefore lombricine, is synthesized from D-serine drawn selectively from the amino-acid pool.

A full account of this work will be published elsewhere.

H. Rosenberg
A. H. Ennor

Department of Biochemistry,
John Curtin School of Medical Research,
Australian National University,
Canberra.

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## Attachment of Immature Simuliidae to other Arthropods

Among biting flies of the family Simuliidae there are several species which, in their early stages, live in a remarkable association with other animals[1,2]. The larvæ and pupæ of the *Simulium neavei* complex, which includes an important vector of human onchocerciasis, are attached to freshwater crabs, and those of the *S. copleyi* complex to mayfly larvæ. The study of the association was interrupted by the extermination of *S. neavei* in a large area of Kenya[3], and the place of oviposition, the way in which the larvæ find their hosts, and the exact effect of the phenomenon are not yet known. The interest of the association is enhanced by its wide distribution; the *S. neavei* complex is represented in the Cameroons Republic[4] as well as in eastern and central Africa, and an unrelated species is associated with mayfly larvæ in the U.S.S.R.[5]. We now record the finding of Simuliids on dragonfly larvæ.

In January about forty crabs were examined near Salisbury, Southern Rhodesia, for larvæ of the *S. neavei* complex but without success. Seven dragonfly larvæ were collected in the Unwindsi stream, and a patch of Simuliid eggs was found on the abdomen, just behind the wing pads, of one of them which was 36 mm. long and belonged to a species of *Aeshna*, probably *rileyi* Calvert. The three hundred or so yellow eggs were placed in a wet tube and they hatched three days later after a journey by air. In the same district a small Simuliid larva was found on the side of a crab in the Ruwa stream. The hypostomium of this larva and first-stage larvæ from the eggs had three prominent apical teeth and was quite unlike that of the *S. neavei* complex, but resembled that of *S. lumbwanus* De Meillon. This character, combined with the presence of ventral papillæ, showed that the larvæ could be *S. adersi* Pomeroy, which was found in the larval stage on stones in the same stream, has a wide range of breeding places, and is probably related to the *S. neavei* complex. Two larvæ of another species, *S. medusaeforme* form *hargreavesi* Gibbins, probably in the third stage, were seen on the frons and the left wing pad of an 18 mm. Libellulid dragonfly larva which was found among a dense mass of these larvæ in the River Parmasea near Lokoja in Nigeria in August

1959. Other larvæ were found in similar circumstances in the River Hawal in the same country.

It seems unlikely that the eggs were laid on the larva by chance; but the significance of their presence is unknown. The finding on aquatic animals of larvæ of two species, at least one of which is outside the *S. neavei* complex and normally attaches itself to inanimate objects, may indicate how the Simuliid–crab association arose. Possibly larvæ attached themselves to crabs, received protection from certain natural hazards, and gave rise to a form which always behaved in this way. Whatever the reason for the findings, they suggest that it would be worth while to examine dragonfly larvæ and other aquatic animals in various parts of Africa and elsewhere in the hope of gaining more information about the association of immature Simuliids with larger animals. Young larvæ should, if possible, be kept alive in running water until they are large enough to be identified.

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D. J. Lewis

Scientific Staff,
Medical Research Council,
c/o British Museum (Natural History),
London, S.W.7.

E. T. Reid

Malaria and Bilharzia Laboratory,
Salisbury,
Southern Rhodesia.

R. W. Crosskey

Commonwealth Institute of Entomology,
London.

J. B. Davies

Ministry of Health,
Kaduna,
Nigeria.

March 17.

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## Inhibition of Nodule Bacteria by an Antibiotic from Legume Seed Coats

The loss of viability of *Rhizobia* on the surface of some legume seeds was described in early reports quoted by Fred, Baldwin and McCoy[1] and more recently by other workers. Greenwood (personal communication) found that survival of *Rhizobia* was better when applied in a peat-pumice carrier than in a milk suspension, and suggested that the white clover (*Trifolium repens*) "seed surface may have a toxic effect on rhizobia". Vincent[2] showed that survival on glass beads was superior to that on subterranean clover (*T. subterraneum*) seed. Lobb[3] suggested that an inhibitory substance may be present in lucerne (*Medicago sativa*) and white clover seed coats. However, in no case was it conclusively demonstrated whether reduced viability was due only to unsuitable physical conditions for survival or also to an inhibitory effect of the seed. Inhibition of organisms other than *Rhizobia*, by antibiotic substances in the seed of flowering plants, has been demonstrated by several workers[4–6].

Table 1. Inhibition of *Rhizobium trifolii* Strain *TA*1 on Plates by Seed and Seed Extracts of *Trifolium subterraneum* L. var. Tallarook

| Seed treatment | Inhibition zones: Mean distance – seed edge to zone edge (mm.) |
|---|---|
| Untreated | 4·1 |
| Autoclaved | 4·7 |
| Soaked and dried | 0·1 *l.s.d.* 5 per cent = 3·4 |
| Soaked, dried, and autoclaved | 0·6 |
| Autoclaved, soaked and dried | 0·9 |
| Autoclaved, soaked, dried and autoclaved | 0·3 |

| Source and treatment of water extract absorbed by filter paper disks | Mean distance – disk edge to zone edge (mm.) |
|---|---|
| Control—no extract | 0·0 |
| Extract of untreated seed | 6·8 |
| Extract of autoclaved seed | 5·2 *l.s.d.* 5 per cent = 3·4 |
| Extract of untreated seed autoclaved after absorption | 4·0 |

Preliminary investigations showed that untreated and autoclaved seed of subterranean clover, and their extracts, inhibited *Rhizobia* in culture, but soaked seed did not. The following experiment was then carried out, using the direct plate method for testing seed[7] and the filter paper disk method for testing extracts[8].

Seed and seed extracts of *Trifolium subterraneum* L. var. Tallarook were treated as listed in Table 1. For soaking, 20 seeds were placed in 10 ml. of distilled water for 6 hr. This water was replaced with 10 ml. for a further 1 hr. before being poured off and the seed dried. Seed extracts were obtained by soaking 400 seeds in 10 ml. sterile water for 7 hr. Sterile filter paper disks (5 mm. diam.) were then immersed for 1 min., removed and dried. Disks were treated 3 times. Seed and filter paper disks were dried, at 50° C. in a sterile oven, to minimize variations between treatments due to differential uptake and loss of water during preparation of the treatments. Autoclaving of seed and extracts (15 lb. per sq. in. for 20 min.) prevented germination of seed, and antibiotic production by seed-borne organisms. Seed soaking and autoclaving treatments were combined to test whether any antibiotic from autoclaved seed formed only as a result of autoclaving was extractable, and, if so, whether it affected *Rhizobia* similarly to that from untreated seed.

Petri dishes, each containing 20 ml. of 1·4 per cent yeast mannitol agar, were surface inoculated with a suspension of $5 \times 10^8$ organisms (*Rhizobium trifolii* strain *TA*1). Three seeds or disks of each treatment were placed on the agar surface of each of 3 plates. Inhibition measurements were made after 93 hr. incubation at 27° C.

Inhibition was indicated by a clear zone, containing no visible colonies, surrounded by a zone of reduced colony size and number. Beyond these zones *Rhizobium* growth appeared normal. The distance of seed or disk edge to the zone of normal growth was measured.

Untreated seed was inhibitory. With soaked seed inhibition zones were very small but a seed extract strongly inhibited growth. Autoclaving of seed did not significantly affect the strength of inhibition whether the seed had been previously soaked or not, showing that no secondary inhibitory substances were formed due to autoclaving. Similarly autoclaving of the seed extract did not affect inhibition. The inhibitory substance in autoclaved seed was extractable with water and the extract of untreated seed retained its activity after autoclaving.

Further investigations showed that the antibiotic was associated with, and extractable from, the seed coat and not the embryo. Contrary to Ferenczy's

findings[4], germination was not necessary for antibiotic production. In fact, recolonization of the inhibited zone was more rapid with viable germinating seeds than with autoclaved seeds.

Some variation occurred between individual seeds but variation between species was more marked. Autoclaved seed of three separate samples of three forage legume species were tested with *Rhizobium trifolii* strain *TA*1. Inhibition was strong with subterranean clover (mean 5·8 mm.), considerably less with white clover (1·5 mm.), and weak with lucerne (0·3 mm.). Differences in seed size do not adequately explain these differences, as lucerne seed is larger than that of white clover.

Field evidence, suggesting the presence in subterranean clover seed of substances inhibitory to *Rhizobia*, has been obtained at this laboratory. Spencer (personal communication) found that soaking of seed prior to inoculation and sowing resulted in greatly improved nodulation from the applied inoculum. On the other hand, when inoculum was applied to the soil there was no beneficial effect of soaking seed. I have found that physical separation of the seed coat and inoculum, by coating the seed with inert material before inoculation, improved nodulation of subterranean clover.

The results presented indicate that the seed coats of the subterranean clover investigated contain a thermo-stable, water-soluble antibiotic, active against a strain of *Rhizobium* normally incorporated in commercially prepared inocula. This substance could contribute to loss of viability of nodule bacteria on the seed both in storage and in field sowings.

J. A. Thompson

Division of Plant Industry,
Commonwealth Scientific and Industrial
Research Organization,
Armidale,
New South Wales.

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## Effect of Autumn Applications of Potassium Gibberellate on Fruit Production of the Strawberry

In the autumn of 1957 at New Brunswick, New Jersey, single-row, 30-ft. replicated plots of Sparkle strawberries were treated with 20 and 50 p.p.m. of potassium gibberellate. Three applications were made, with the first application applied on September 13. An additional variable was added by using both a 1-week and 2-week interval between spray applications. No effect was noted in the autumn of 1957 from these sprays except for an occasional inflorescence observed on the plots sprayed with 50 p.p.m. The next spring the sprayed plots had a heavier bloom early in the flowering period as compared with the unsprayed plots. The harvest records for 1958 showed that the treated plots produced about a 30 per cent larger volume of early fruit than the unsprayed checks, with no difference due to interval between spray applications. Due to rains and fruit rots during the latter part of the 1958 harvest season, total yield records were not recorded.

In the autumn of 1958 another investigation was conducted using 30-ft. single-row plots of Sparkle strawberry plants. Each treatment was replicated five times. Treatments consisted of 0, 10, 20, 30, 40, 50 and 100 p.p.m. of potassium gibberellate (supplied by Merck and Co., Rahway, New Jersey, which supported this work with a grant-in-aid) applied as sprays on September 17, October 1 and October 15. Some inflorescences were observed later in the autumn on plots sprayed with 40, 50 and 100 p.p.m., and petiole elongation was observed at the 100 p.p.m. level.

Table 1. Effect of Potassium Gibberellate Sprays on Yield of Sparkle Strawberry Fruit

| Spray concentration (p.p.m.) | Yields | |
|---|---|---|
| | Early harvest* (quarts/acre) | Total† (quarts/acre) |
| 0 | 2,603 | 10,662 |
| 10 | 3,758 | 9,519 |
| 20 | 3,616 | 8,637 |
| 30 | 3,515 | 8,252 |
| 40 | 2,485 | 5,209 |
| 50 | 2,390 | 5,241 |
| 100 | 832 | 1,673 |
| *L.S.D.* 0·05 | 958 | 1,882 |
| *L.S.D.* 0·01 | 1,298 | 2,550 |

* Includes harvests of May 28, June 2 and June 5.
† Total yield was based on above three harvests plus an additional five completed by June 26, 1959.

The results obtained in the 1959 harvest season (Table 1) indicate that by June 5 (including three harvests) the 10 and 20 p.p.m. treatments had significantly increased the volume of fruit harvested as compared with the unsprayed checks: the 100 p.p.m. treatment, on the other hand, had significantly reduced yields. The total yields (including eight harvests) for these plots showed that there was no significant difference between the unsprayed checks and the 10 p.p.m. treatment, while all the other treatments showed a significant reduction in yield.

The application of three sprays of 10 p.p.m. of potassium gibberellate in the autumn may give the strawberry grower an economic advantage, since the early fresh market fruit commands a premium price.

Carter R. Smith

Department of Horticulture,
Rutgers—The State University,
New Brunswick,
New Jersey.

## Viability and Germination Inhibitor of the Seed of Rice

The loss of viability of seeds within one year after harvesting has been reported by Grist[1] from varieties of rice grown in British Guiana and by Ramiah[2] in India, but no explanation has been offered. Germination tests of a winter variety 'Rupsail' recently carried out in this laboratory indicated peak germination

Table 1. Respiration-Rates of Rice Seeds without Husk

| Year of collection | $\mu$l./$O_2$/hr./mgm. dry weight | $\mu$l./$CO_2$/hr./mgm. dry weight |
|---|---|---|
| 1953 | 0·078 | 0·054 |
| 1957 | 0·078 | 0·080 |
| 1958 | 0·142 | 0·113 |

Table 2. Root and Shoot Growth (cm.) in Solutions leached from Seeds of Rice harvested in Different Years

| Growth (hr.) | Control (distilled water) | | 1958 | | 1957 | | 1953 | |
|---|---|---|---|---|---|---|---|---|
| | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot |
| 24 | 0·25 ± 0·02 | 0 | 0·23 ± 0·02 | 0 | 0·26 ± 0·04 | 0 | 0·15 ± 0·01 | 0 |
| 48 | 3·08 ± 0·12 | 1·08 ± 0·30 | 3·50 ± 0·52 | 0·83 ± 0·14 | 3·45 ± 0·24 | 1·36 ± 0·12 | 1·83 ± 0·30 | 0·93 ± 0·18 |
| 72 | 4·18 ± 0·36 | 1·94 ± 0·21 | 5·89 ± 1·10 | 2·58 ± 0·23 | 4·98 ± 0·14 | 2·33 ± 0·48 | 4·02 ± 0·37 | 2·28 ± 0·07 |
| 96 | 6·28 ± 0·83 | 3·54 ± 0·45 | 7·25 ± 0·26 | 2·77 ± 0·29 | 6·38 ± 0·37 | 4·68 ± 0·46 | 4·89 ± 0·97 | 3·72 ± 0·31 |
| 120 | 9·76 ± 0·12 | 8·30 ± 1·0 | 9·88 ± 0·46 | 7·88 ± 0·80 | 7·33 ± 0·88 | 13·0 ± 2·80 | 5·10 ± 0·05 | 10·2 ± 0·41 |
| 144 | 11·20 ± 0·57 | 8·53 ± 0·53 | 12·23 ± 0·73 | 9·20 ± 1·40 | 8·33 ± 0·17 | 14·56 ± 0·93 | 5·36 ± 0·33 | 13·03 ± 1·70 |
| 168 | 15·43 ± 0·96 | 12·01 ± 1·10 | 12·43 ± 0·75 | 15·36 ± 0·95 | 8·90 ± 1·00 | 16·50 ± 0·76 | 5·56 ± 0·20 | 17·20 ± 1·90 |

Table 3. Effect of Light Qualities on the Percentage of Germination of Rice Seeds

| | White | Blue | Green | Yellow | Red | Dark | Far-red | 12-hr. period of white light + dark | 8-hr. period of white light + red + dark | 12-hr. period of red + far-red | 12-hr. period of far-red + red |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 24-hr. exposure of soaked seeds | 98·10 ±0·50 | 92·30 ±1·02 | 94·95 ±0·05 | 88·85 ±1·02 | 85·30 ±2·00 | 87·95 ±0·02 | 78·30 ±0·83 | 98·40 ±0·03 | 60·80 ±0·40 | 45·34 ±0·19 | 45·06 ±0·25 |

during March–June following harvesting in December; thereafter it declined and from November onwards the viability was lost. Seeds harvested in 1953 and 1957 failed to germinate in 1959. The excised embryos from fresh seeds of 1958 germinated only in White's nutrient agar media while those of one- or five-year old seeds did not grow. Respiration-rates measured by Warburg apparatus indicated higher rates in fresh seeds than the old ones (Table 1).

The results thus show that the failure of germination of old seeds of 1953 and 1957 is not due to the nature of the husk acting as a barrier with increasing carbon dioxide concentration inside or the old seeds becoming dead. The possibility of the presence of germination inhibitor and its formation was then investigated.

Tests were made on the basis that the inhibitor, if present, might be leached out of the seeds during soaking in water for 24 hr. Leachings from seeds of 1953, 1957 and 1958 were therefore used for the growth of viable seeds; 88–90 per cent seeds just sprouted but subsequent growth of root was inhibited in the leachings from the non-viable seeds of 1953 and 1957 and shoot growth accelerated in later periods (Table 2). Promotion of growth of the shoot and inhibition of growth of the root are suggestive of auxin action. Auxin assay of the embryo extracts by root inhibition tests showed that embryos from old seeds contained a higher amount of indoleacetic acid equivalent in comparison to that of viable seeds. The concentration was much increased in the embryos of 5-year-old seeds. From consideration of the presence of a large amount of auxin in the embryos of non-viable seeds and reduction of embryonic root growth in the leachings, it is presumed that there exists a close relation between auxin concentration and the formation of germination inhibitor.

Experiments performed on the effects of different light qualities indicated that white light increased germination over the percentage in the dark. But the percentage in red was not increased over darkness while far-red rays had the largest inhibiting effect (Table 3). In photoblastic seeds Toole *et al.*[3] have recorded stimulation in red over darkness, but infra-red following red inhibited germination. Inhibiting effect in rice seed was further shown when red was interpolated between white light and dark as the percentage of germination was considerably reduced in comparison with treatments of light and dark. It appears that the absorption of red and far-red rays leads to the formation of germination inhibitor in rice seeds.

We thank Miss Protima Datta Ray for assistance in the measurement of respiration-rates.

S. M. Sircar
Manjula Biswas

Department of Botany,
University of Calcutta,
Calcutta, 19.
March 18.

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## Foliar Application of Nutrients and Rhizosphere Microflora of *Camellia sinensis*

Susceptibility of plants to fungi causing root-rot is known to be conditioned by the rhizosphere microflora[1,2]. Changes in this microflora can result from derangement in plant metabolism caused by either foliar treatment with chemicals[3], fungal infection of the roots[3] or even systemic infection by plant viruses[4]. Therefore, it seems possible to stimulate artificially the multiplication of specific micro-organisms in the rhizosphere by selective plant treatment, such as foliar application of nutrients, designed to act directly on the plant without affecting the soil. Work on these lines, apart from a recent report[5], has received little attention. Observations on sprayed tea plants reported here support the basic concept of changing the pattern of the rhizosphere microflora by foliar sprays.

One-year-old monoclonal selections of tea plant, propagated from cuttings, were grown in tea field soil in earthenware pots, and each experimental plant was sprayed with an atomizer with 10 ml. aliquots of nutrient solutions (Table 1). Distilled-water sprayed plants formed controls. The soil was protected from run-off spray by a plastic cover. The plants were kept under a bell-jar for 24 hr. after each spray application, four of which were made at 2-day intervals. Four days after the completion of the last spray, rhizosphere soil from each treatment (three replicates) was sampled and dilutions plated in soil-extract, glucose, nitrate-agar for bacterial, and in peptone, dextrose-agar containing 1 : 10,000 crystal violet and 15 μgm./ml. each of streptomycin and dihydrostreptomycin for fungal, counts.

Table 1

| Foliar spray treatments | Rhizosphere population in 1 gm. dry soil | | |
|---|---|---|---|
| | Fungi (in thousands) | Bacteria (in hundred thousands) | Fungal genera and species occurring in the rhizosphere |
| (*A*) 0·76 *M* $(NH_4)_2SO_4$ | 125·0 | 32·3 | (1), (2)* |
| (*B*) 0·04 *M* $MgSO_4.7H_2O$ | 93·5 | 12·3 | |
| (*C*) 0·67 *M* KCl | 256·5 | 27·0 | (2) |
| (*D*) 1·47 *M* $NaNO_3$ | 176·5 | 33·0 | (2) |
| (*E*) 0·28 *M* $Na_2HPO_4.12H_2O$ | 281·5 | 14·0 | (2), (3) |
| (*F*) 0·83 *M* Urea | 73·5 | 20·3 | (1) |
| (*G*) Control: distilled water | 193·5 | 44·0 | (2) |
| Difference required for significance, $P = 0·01$ | 32·0<br>FBADGCE | 6·3<br>BEFCADG | |

* (1) *Stysanus* sp., (2) *Scopulariopsis brevicaulis* Bain., (3) *Myrothecium* sp.

Foliar spray with any of the inorganic or organic nutrients significantly reduced the bacterial population of the rhizosphere soil (Table 1). 0·67 *M* solution of potassium chloride and 0·28 *M* disodium hydrogen phosphate significantly increased the number of fungi in the rhizosphere soil over that in the control, whereas a significant reduction of fungal numbers was recorded with application of 0·04 *M* magnesium sulphate. The rhizosphere fungi responded differently to spray containing comparable amounts of nitrogen but from different nitrogen sources. With 1·47 *M* solution of sodium nitrate there was no significant difference in fungal numbers over control plants, but spraying with 0·76 *M* ammonium sulphate and 0·83 *M* urea significantly decreased the fungal population. Although spray application with urea markedly increased fungal population in the rhizosphere of rice plants[5], the reverse seems to be the case in tea plants.

Table 1 also summarizes specific differences in the genera and species of fungi occurring in the rhizosphere of plants sprayed with different nutrilites. Such differences in the occurrence of a species of *Penicillium* in the rhizosphere of urea-sprayed rice plants has recently been reported[5]. Roots of plants subjected to high temperature, high light intensity[6] or desiccation[7] are known to exude large amounts of amino-acids and these energy sources are presumed to be used up by micro-organisms[8]. Differences in the rhizosphere microflora resulting from foliar application of nutrients is probably due to qualitative and quantitative changes in the root exudates induced by the plant treatment. Further work on these lines is in progress and details will be published elsewhere.

I thank Prof. T. S. Sadasivan for helpful suggestions, the Director of the Commonwealth Mycological Institute, for identification of fungal cultures and the Chief Scientific Officer of this Department for facilities to carry out this investigation.

C. S. Venkata Ram

Tea Experiment Station,
P.O. Devarshola,
South India.
March 7.

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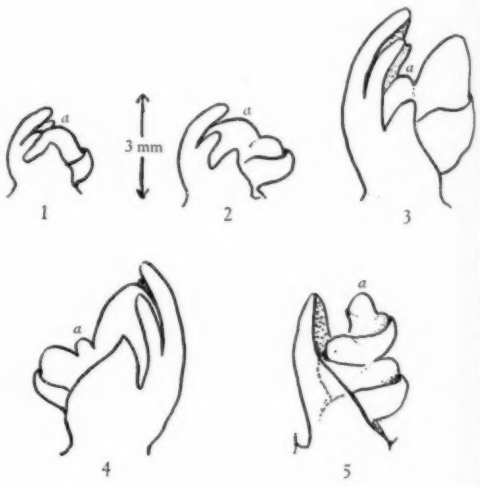
## Axial Origin of the Terminal Inflorescence in the Genus *Trifolium*

J. L. Stoddart[1] states that floral initiation in red clover begins by the enlargement of the shoot apex. The following observations show that the actual shoot apex is absorbed by an adjoining lateral bud. The origin of the terminal inflorescence of this species as a lateral bud is therefore similar to that of the axillary inflorescence in other species.

In species with axillary inflorescences (for example, *T. subterraneum*[4] and *T. repens*), the change from the vegetative state (Fig. 1) to the reproductive begins at the shoot apex (*a*) with the formation of a bulge in the axil of the topmost leaf initial (Fig. 2). A similar bulge is formed in each further leaf initial, the bulge and leaf initial developing into the type of inflorescence and leaf typical of the species, and the tissue expanding between each leaf to make the lengthened internodes characteristic of the flowering stage of the shoot.




In species with terminal inflorescences (for example, *T. pratense* and *T. alexandrinum*), the reproductive state begins in the same way, but is followed by domination and absorption of the shoot apex by the first one or two lateral bulges. Usually the first bulge is the only one; it increases in size (Fig. 3) until the shoot apex disappears. Florets are differentiated from a series of small bulges formed over its surface. Sometimes a second bulge is formed in the axil of another leaf initial before the shoot apex stops growth. In this case, the apex can be seen for a short time between the two enlarging inflorescence primordia (Fig. 4). The younger inflorescence is usually the smaller of the two when fully developed.

It is interesting to note that the terminal inflorescences of well-known gramineous genera also originate from lateral bulges in leaf initials close to the shoot apex (for example, oat, Fig. 5). Apart from differences in floret structure peculiar to each family, the mature inflorescence of a grass or cereal therefore differs from that of a clover mainly by the number of 'fertile' leaf initials, and by the development of each such leaf initial to form a small ridge

only instead of a leaf (for example, *Avena sativa*[2] and *Lolium perenne*[3]).

YVONNE AITKEN

School of Agriculture,
University of Melbourne.

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## BACTERIOLOGY

### A Growth Factor for *Haemophilus* Species secreted by a Pseudomonad

THE clustering of bacterial colonies of one species around those of another—satellitism—was first described for *Haemophilus influenzae* by Grassberger in 1897[1]. The interrelationship is probably due to the supply of critical nutrients by one micro-organism to the other. While the special nutritional requirements of *Haemophilus* species have been studied and some compounds essential for growth identified[2], the nature of bacterial substances responsible for the satellite phenomenon have not been investigated. In this communication we report on the isolation of such substances.

It is much easier to study excretory products of bacteria in a chemically defined medium than in a complex one. Therefore, only cultures able to grow in simple media were screened for their ability to stimulate growth of *Haemophili* on blood agar or support it on brain heart infusion (Difco Laboratories, Detroit) agar. Test organisms included seven strains of *Haemophilus* of animal origin, four strains of *H. influenzae*, and one strain each of *H. parainfluenzae*, *H. parahaemolyticus* and *H. gallinarum*. The cultures were streaked on test media and crossed with a single streak of the possible 'feeder'. A good 'feeder' was found to be a pseudomonad isolated from an emperor penguin and obtained from Dr. L. A. Page. This bacterium grew well in a solution of histidine (200 mgm. per cent), monobasic potassium phosphate (20 mgm. per cent), mineral mix No. 49 (supplied by Dr. J. H. Hunter, Haskins Laboratories, New York, and containing iron, manganese, zinc, cobalt, copper, vanadium and boron) (8 mgm. per cent) at pH 7·5 and 37° C. When grown in such a medium, the organism secreted growth factors essential for *Haemophilus* species, apparently the same as produced in the more complex media.

The penguin pseudomonad was grown in 1 litre of the above medium for 48 hr. After the cells had been removed by centrifugation, the supernatant was Seitz-filtered and lyophilized. The dried material was redissolved in 10 ml. of water and chromatographed, using ascending chromatography with a solvent system consisting of butanol, acetic acid, and water (4 : 1 : 5). Two ultra-violet-absorbing spots were observed under illumination with a 'Mineralight' (2537 *A*) (Ultra-violet Products, Inc., South Pasadena), of which one was much denser than the other. An additional spot was detected after spraying with ninhydrin—probably histidine from the growth medium, as shown by a reference sample.

The ultra-violet-absorbing material (*EP*) contained in the denser spot was eluted from the paper and added to brain heart infusion agar. This medium could then support the growth of *Haemophili*. *EP* was further purified by repeated partition on Whatman No. 1 filter paper and subsequent elution. The solvents, used in sequence, were: monobasic sodium phosphate, 5 per cent; *iso*propanol, ethanol, and water in the ratio of 7 : 2 : 1; and *iso*propanol and 0·2 *M* ammonium hydroxide in the ratio of 3 : 1. Prior to use, the filter paper had been washed for 24 hr. in distilled water.

Purified *EP* had ultra-violet absorption optima at 265 and 275 mμ at *p*H 1·0 and 11·0, respectively. On hydrolysis, with 2 *N* hydrochloric acid at 115° C. for 60 min., a carbohydrate was released which, in three different solvents, had the same $R_F$ as ribose. *EP* was found to be free of phosphorus. In aqueous solution it could be heated at 100° C. for 30 min. and 120° C. for 5 min. without substantial loss of activity; heating at 120° C. for 10 min. resulted in complete inactivation.

Species of *Haemophilus* require for growth either *V* factor—replaceable with diphosphopyridine nucleotide, or its nucleoside[3]—*X* factor—replaceable with hæm, or both. Since most of our animal strains which were stimulated by *EP* showed no hæm requirement, a relationship between *EP* and diphosphopyridine nucleotide was suggested. *EP*, both before and after acid hydrolysis, had a different $R_F$ value from niacin, niacinamide, niacinamide riboside, niacinamide mononucleotide, and diphosphopyridine nucleotide. It also differed from these in ultra-violet absorption spectra. Infra-red absorption spectra do not exclude *EP* from being a derivative of niacinamide.

*EP* could be replaced as a growth factor for *Haemophilus* by diphosphopyridine nucleotide and niacinamide mononucleotide but not by niacinamide, adenosine triphosphate or the products of acid hydrolysis of ribo- or deoxyribo-nucleic acids. Test organisms grew in brain heart infusion broth with either niacinamide mononucleotide or diphosphopyridine nucleotide in the concentration of 10γ per cent, whereas *EP* was required at the concentration of 1,000γ per cent. While it is possible that this amount reflects the true needs of the test species, the possibility of partial inactivation of the compound during the purification process cannot be disregarded. Dorfman *et al.*[4], working with *Shigella*—which requires nicotinic acid or niacinamide for growth—showed that N-substituents of niacinamide served as growth factors while substitution on the ring inactivated the molecule. In one case where quinolinic acid could be used to promote some growth, its effective concentration was 1,000 times that of niacinamide.

While the data on hand are insufficient to characterize *EP* completely, present information suggests that it might be a niacinamide riboside with unknown substitutions. Its implied biological significance would be consistent with such an interpretation. Work to elucidate further the structure and composition of *EP* is in progress.

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MOSHE SHIFRINE
ERNST L. BIBERSTEIN

School of Veterinary Medicine,
University of California,
Davis, California.

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## Antibody Responses of the Domestic Fowl to Two Soluble Bacterial Antigens

For some years now virologists have recognized the value of the domestic fowl as a means of obtaining high-titred antisera to viral and rickettsial antigens. Bacteriologists and immunologists have almost invariably preferred the rabbit, consequently little information is available about the responses of fowls to soluble bacterial antigens. While investigating the immunological tolerance of diphtheria alum-precipitated toxoid in the domestic fowl[1] I had the opportunity of collecting a limited amount of information about the primary and secondary antibody responses of White Leghorn fowls to both tetanus alum-precipitated toxoid (Wellcome series *W6874A*) and diphtheria alum-precipitated toxoid (Wellcome series *BA6403A*); though only one dosage of each antigen was used, the results may be of value to other workers.

Twenty birds between the ages of 10 and 14 months were used in the experiment. One group of ten was given a primary stimulus of 1·0 ml. diphtheria alum-precipitated toxoid ($20L_f$) intramuscularly; the other received 1·0 ml. tetanus alum-precipitated toxoid ($50L_f$) by a similar route. A sample of blood was taken from each bird immediately before primary challenge and then at 3- or 4-day intervals for 31 days. A second dose (1·0 ml.) of the respective antigens was given intramuscularly 34 days after the last bleeding (that is, 65 days after primary challenge). The fowls were bled immediately before this injection and then at intervals of 2 or 3 days for the next 15 days.

The serum was separated from these blood samples by centrifugation and the appropriate samples tested for their diphtheria antitoxin content (Romer and Sames[2], as modified by Glenny and Llewellyn-Jones[3]), or their tetanus antitoxin content[4].

Fig. 1 shows the average primary and secondary antibody response of ten fowls to diphtheria alum-precipitated toxoid. Antibody to this antigen was

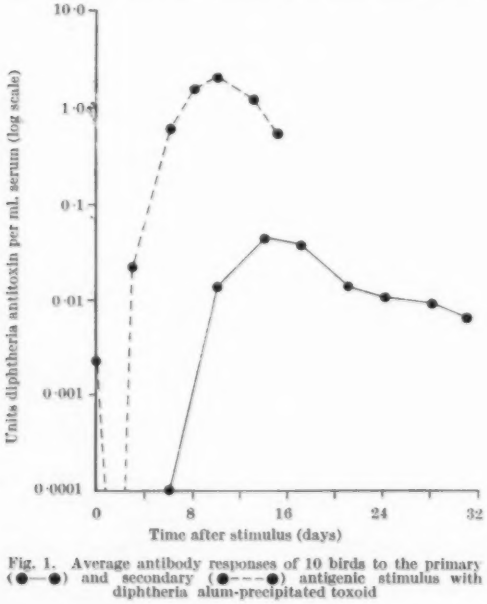


Fig. 1. Average antibody responses of 10 birds to the primary (●—●) and secondary (●- - -●) antigenic stimulus with diphtheria alum-precipitated toxoid

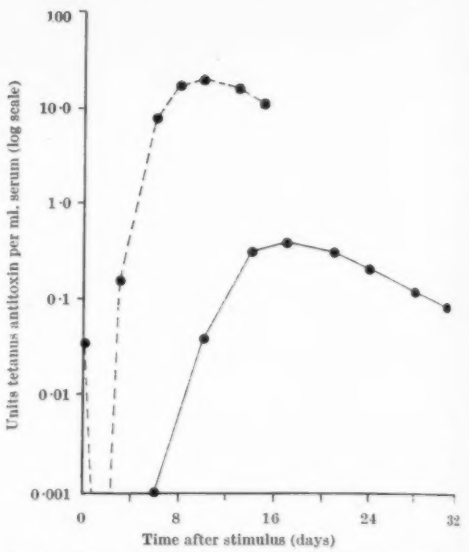


Fig. 2. Average antibody responses of 10 birds to the primary (●—●) and secondary (●- - -●) antigenic stimulus with tetanus alum-precipitated toxoid

first detected 10 days after primary stimulation, reached a maximum in most cases on the fourteenth day and then gradually declined. After secondary stimulation, antibody was always detectable after 3 days and reached maximum concentration between the eighth and thirteenth day, the tenth day after injection being the most usual.

Fig. 2 shows the primary and secondary antibody responses to tetanus alum-precipitated toxoid. These responses are similar in form to those with diphtheria alum-precipitated toxoid as the antigen, except that the maximum response to primary challenge with tetanus alum-precipitated toxoid appears to be delayed for about three days and is therefore not usually reached until the seventeenth day. This finding was general among the group.

The individual maximum antibody concentrations reached were 0·1 I.U. diphtheria antitoxin and 3·0 I.U. tetanus antitoxin per ml. of serum during the primary response, and during the secondary response 5·0 I.U. diphtheria antitoxin and 65 I.U. tetanus antitoxin. There was, of course, considerable individual variation in the amounts of antibody produced, but, as can be seen from the figures, the antibody responses in these birds compare favourably with those obtainable in the rabbit under similar conditions.

A number of fowls were also injected with both antigens simultaneously[1], one antigen being injected into each leg. Results similar to those described above were obtained and there was no evidence of any interference between the two antigens.

G. Gowland

Department of Bacteriology,
School of Medicine,
Leeds, 2.
March 17.

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# FORTHCOMING EVENTS

## Tuesday, August 16

INSTITUTE OF METALS (at 17 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. J. Gilman: "The Physical Nature of Plastic Flow".

# APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LIBRARIAN (with a university degree)—The Secretary, The University, Aberdeen (August 20).

RADIO PROPAGATION ENGINEER (with an appropriate university degree with honours or equivalent, and experience in radio propagation research and meteorological physics) at the Research Laboratories, Australian Post Office, Melbourne, for the scientific investigation of tropospheric radio propagation as related to general meteorological phenomena—The Australian Post Office Representative, Australia House, Strand, London, W.C.2 (August 20).

RESEARCH ASSISTANT, to perform theoretical investigations on various aspects of semiconductors—Prof. P. T. Landsberg, Department of Applied Mathematics, University College, Cathays Park, Cardiff (August 20).

UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF VETERINARY CLINICAL STUDIES, to assist in the teaching of clinical pathology—Dr. G. A. Gresham, The Medical School, The University, Tennis Court Road, Cambridge (August 25).

SENIOR LECTURER IN MATHEMATICS; and a SENIOR LECTURER IN MATHEMATICAL STATISTICS at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, August 31).

RESEARCH ASSISTANT (PLANT BIOCHEMIST) to Dr. B. T. Cromwell, for work on biosynthesis of alkaloids in higher plants—The Registrar, The University, Hull (September 3).

LECTURER (ELECTRON MICROSCOPY), for integrated biochemical and cytological studies of cell division; and a LECTURER (PHYSICAL BIOCHEMISTRY), for studies of proteins, particularly of wheat grain, IN THE DEPARTMENT OF AGRICULTURAL CHEMISTRY at the Waite Agricultural Research Institute, University of Adelaide, Australia,—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 5).

BOTANIST, Scientific Officer grade (with a good honours degree and preferably postgraduate training or research experience), to study the development and anatomy of apples in relation to storage behaviour—The Secretary, East Malling Research Station, near Maidstone, Kent (September 7).

LECTURER (graduate in civil, mechanical or electrical engineering) IN ENGINEERING; and a LECTURER IN GEOLOGY at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (September 15).

LECTURER/SENIOR LECTURER (qualified in grassland management) IN ANIMAL HUSBANDRY at the University of Sydney Animal Husbandry Farms, Camden, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 15).

RESEARCH BIOPHYSICIST (with a degree in medicine or biology, previous research experience, and preferably experience in the technique of electron-microscopy), to undertake an investigation into some aspects of industrial chest disease with special reference to asbestos, and to work either in the Department of Pathology or in the Cavendish Laboratory, or partly in both Departments—The Secretary of the Department of Physics, The Cavendish Laboratory, The University, Cambridge (September 15).

SENIOR LECTURER AND DIRECTOR (with high academic qualifications and experience of high-speed digital computers) OF THE SUB-DEPARTMENT OF COMPUTATION—The Secretary and Registrar, The University, Southampton (September 15).

LECTURER/SENIOR LECTURER and a LECTURER IN PSYCHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 16).

CHAIR OF PSYCHOLOGICAL MEDICINE—The Secretary, The University, Edinburgh (September 23).

CHAIR OF BOTANY in the University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, September 30).

READER IN MATHEMATICS—Prof. Titchmarsh, Mathematical Institute, The University, Oxford (October 15).

SCIENTIFIC OFFICER (honours graduate, preferably with two to three years postgraduate experience) IN THE ENTOMOLOGY DEPARTMENT—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (October 15).

ASSISTANT, Grade B, IN THE ARCHITECTURE AND BUILDING DEPARTMENT, to teach Mathematics to G.C.E. "A" level standard to full-time students in the Land Surveying Section—The Clerk to the Governors, South-West Essex Technical College and School of Art, Forest Road, Walthamstow, London, E.17.

ASSISTANT PRINCIPAL (with a university degree in forestry and preferably experience in teaching and tropical forestry) OF THE FOREST SCHOOL, Uganda—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.61/9/08/C1.

EDUCATION OFFICER (with a university degree or equivalent qualification and several years experience in a special library or information department, preferably in industry), to make a special study of the training requirements of special librarians and information officers and to organize frequent short courses in this field)—The Director, Aslib, 3 Belgrave Square, London, S.W.1.

FIELD SEISMOLOGIST (with an honours degree in mathematics and physics, geophysics or a closely related science, plus experience in seismological field work) IN THE DEPARTMENT OF MINES AND TECHNICAL SURVEYS, Canada, to direct a group conducting long-range seismic observations on the Polar Continental Shelf and at sea with the most modern magnetic tape equipment—Dept. 1510, National Employment Service of Canada, 61 Green Street, London, W.1.

GAME OFFICER (male, with a university degree in zoology) in Kenya, initially for normal Game Warden duties, but after experience of Kenya conditions, for research work—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.152/7/04.

GRADUATE RESEARCH ASSISTANT IN PHYSICS, to work on studies of the structure of diamonds by optical methods—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey.

LECTURER (with a degree in electrical engineering and appropriate professional qualifications, with teaching and industrial or research experience) IN ELECTRICAL ENGINEERING—The Principal, Kingston Technical College, Fassett Road, Kingston-upon-Thames, Surrey.

LECTURERS (2) IN ZOOLOGY, one to teach histology and microtechnique, and the other to teach ornithology (and, if possible, animal behaviour)—MacGill University, Montreal, Quebec, Canada.

METEOROLOGISTS (men or women, with a good honours degree in mathematics and/or physics, and preferably experience in a meteorological service, especially in analysis and forecasting) in the East African Meteorological Department, for duties which may include weather analysis and forecasting (including research); consultative meteorological work related to agriculture, hydrology, etc.; research into climatology, rain stimulation, evaporation control, and work in the field of geomagnetism or solar radiation—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.57/88/01/C1.

PROFESSOR OF PHYSIOLOGY, and a PROFESSOR OF BIOCHEMISTRY at the University of Shiraz, Iran—The Director, Recruitment Department, British Council, 65 Davies Street, London, W.1, quoting N.12/T (60).

RESEARCH ASSISTANT (preferably with an honours degree, some experience of vacuum techniques, electronics, or a knowledge of dielectrics) IN PHYSICS in the Department of Natural Philosophy, for work on the properties of thin dielectric films—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1.

RESEARCH WORKERS (2) (with an honours degree in biochemistry or zoology, and preferably with previous research experience) IN THE DEPARTMENT OF ZOOLOGY, for the extension of fundamental work now in progress on (*a*) the mode of action of insecticides and (*b*) chemoreception of insects—Dr. A. B. P. Page, Imperial College Field Station, Ashurst Lodge, Sunninghill, Ascot, Berks.

REXCO RESEARCH AND DEVELOPMENT COMPANY RESEARCH STUDENTS (with, or anticipating to have, a good honours degree in chemistry, Grad.R.I.C., or a Dip.Tech. in pure or applied chemistry), for the study of products from the low temperature carbonization of coal—Head of the Department of Applied Chemistry, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

SCIENTIFIC OFFICERS or SENIOR SCIENTIFIC OFFICERS (with a first- or second-class honours degree in an appropriate subject or equivalent) at Home Office Forensic Science Laboratories at Cardiff (CHEMIST), Preston (CHEMIST and BIOLOGIST) and Birmingham (CHEMIST or BIOLOGIST), for examination of material received from police and to give evidence in criminal courts—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. F.454/0A.

SENIOR LECTURER IN INORGANIC CHEMISTRY; LECTURERS (2) IN CHEMICAL ENGINEERING; LECTURERS (2) IN ORGANIC CHEMISTRY; an ASSISTANT, Grade B, IN APPLIED CHEMISTRY (DYEING); and an ASSISTANT, Grade B, IN METALLURGY—The Registrar, Bradford Institute of Technology, Bradford 7.

TECHNICAL OFFICER (with a recognized degree or National Diploma in Agriculture, Horticulture or Agricultural Botany) IN THE SEED PRODUCTION BRANCH, to specialize on root and vegetable seed crops—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

# REPORTS and other PUBLICATIONS

*(not included in the monthly Books Supplement)*

## Great Britain and Ireland

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Falkland Islands Dependencies Survey. Scientific Reports. No. 19: The Geology of South Georgia—II. By Dr. A. F. Trendall. Pp. 48+5 plates. 25*s*. net. No. 23: The King Penguin *Aptenodytes patagonica* of South Georgia. 1: Breeding Behaviour and Development. By Dr. Bernard Stonehouse. Pp. 81+7 plates. 25*s*. net. (London: H.M. Stationery Office, 1960.) [235

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Royal Greenwich Observatory Bulletins. No. 18: Photographic Zenith Tube 1959. Pp. 25–50. (London: H.M. Stationery Office, 1960.) 4*s*. net. [235

Transactions of the Royal Society of Edinburgh. Vol. 64, No. 1: Glaucophane-Schist, Eclogite, and Associated Rocks from Knockormal in the Girvan-Ballantrae Complex, South Ayrshire. By Dr. T. W. Bloxam and Dr. J. B. Allen. Pp. 1–27+2 plates. 10*s*. 6*d*. Vol. 64,

No. 2 : On the Structure of *Calymmatotheca kidstoni* Calder (emended) and *Genomosperma latens* gen. et sp. nov. from the Calciferous Sandstone Series of Berwickshire. By Albert G. Long. Pp. 29–44+4 plates. 8*s*. 6*d*. (Edinburgh : Royal Society of Edinburgh, 1960.) [235

Colonial Office. Fishery Publications No. 13, 1960 : The Plankton of Inshore Waters off Freetown, Sierra Leone. By V. Bainbridge. Pp. iv+48. (London : H.M. Stationery Office, 1960.) 35*s*. net. [235

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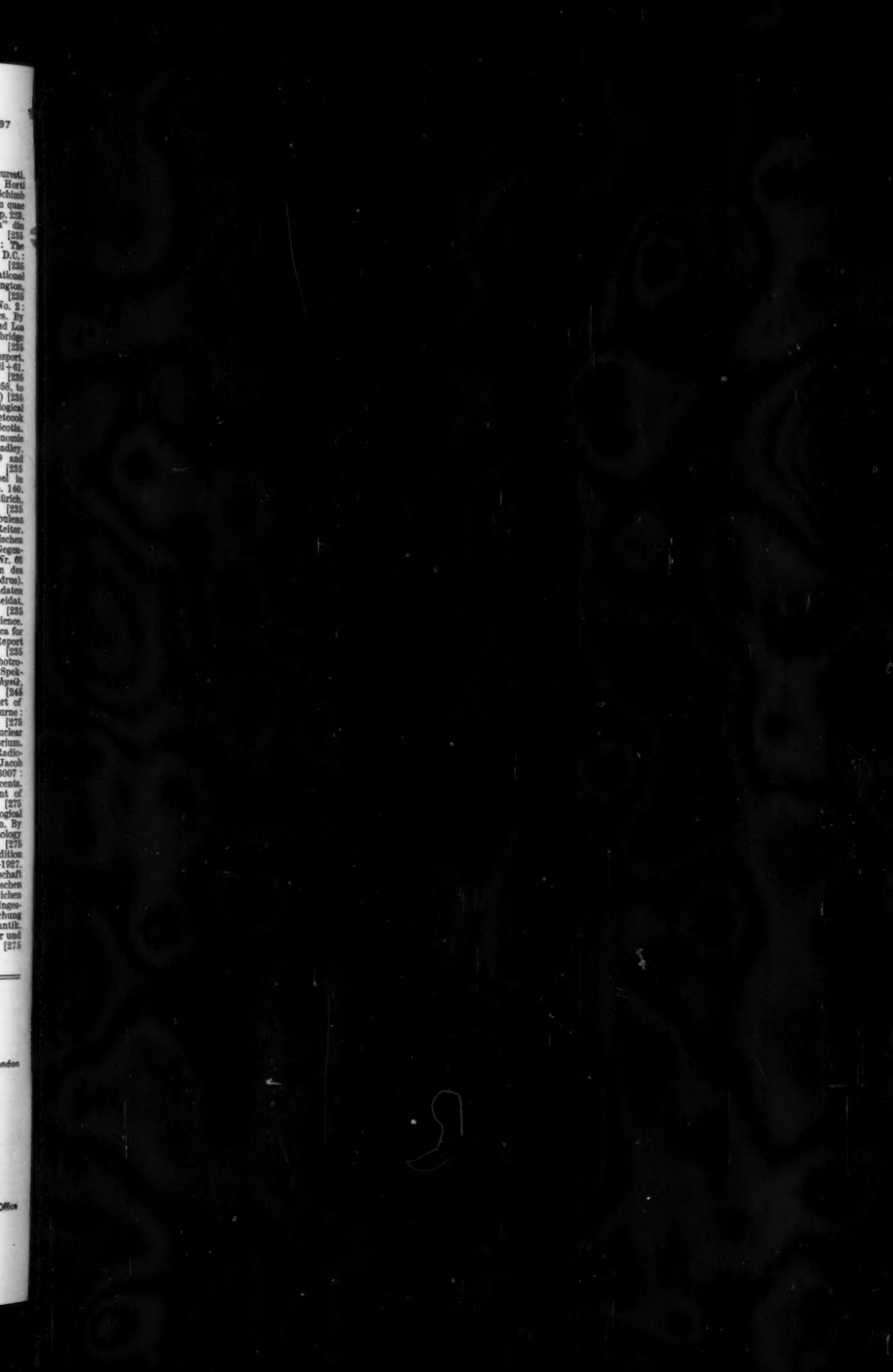
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